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FURTHER STUDIES OF HEPATIC STRUCTURE AND FUNCTION BY FLUORESCENCE MICROSCOPY*

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Earlier publications from this laboratory have been devoted to various aspects of hepatic structure and function in amphibia and mammals which can be visualized by fluorescence microscopy, in the absence of and following the injection of fluorescent compounds which are readily excreted into the bile (1–5). These studies have been concerned primarily with attempts to elucidate the mechanism of biliary excretion under normal, physiological conditions; and it is believed that, in conjunction with those of others, they have afforded an essentially valid account of the normal excretory process.

In the course of our attempts to understand the normal process, we have repeatedly encountered a variety of unusual fluorescent images of both hepatic cells and bile canaliculi. The most striking of these are an extraordinary "sprouting" of vacuoles from the canaliculi, the development of more or less widespread intracellular vacuole formation, and the selective concentration of dyes in the cytoplasm of the hepatic cells. It has been necessary to become sufficiently well acquainted with these aberrant phenomena in order to determine their normal or abnormal character; and special studies have made it clear that they form no part of the normal picture. It is these abnormalities which are the particular subject of the present report. As observed under different conditions, they are in general highly repetitive in character in both frog and mammals. Many of their features are still obscure; and, in the present inadequate state of our knowledge, it has seemed worthwhile to describe each of the distinctive phenomena in terms of our own experience, which differs in certain important respects from that of others. These more general descriptions are supplemented by observations upon the phenomena as they develop following radiation of the liver with ultraviolet light, and following cauterization of its

* We are deeply indebted to Miss Elizabeth H. Bagley, who collaborated in some of the observations and experiments; to Professor Robert W. Wood, for the loan of a prism spectrograph; to Dr. Philip Nolan and Mr. John M. Bodenchuk of the Farrand Optical Co., Inc., for their extremely generous cooperation in making possible accomplishment of the fluorescence emission spectra; to Miss Virginia V. Croker, for secretarial and bibliographic assistance; and to Miss Nancy M. Gray, for technical assistance.

The "Ultropak" microscope and accessories employed were made available on loan from the Armed Forces Institute of Pathology.

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surface. Taken as a whole, these abnormalities constitute a most revealing picture of the distortion of normal hepatic function under varying experimental conditions.

MATERIALS AND METHODS

The characteristics of the fluorescence microscope employed, as well as materials and general methods of procedure, were for the most part as described elsewhere (2). For more efficient absorption of heat from the filtered light beam, the equipment was modified by the use of a much larger water cuvette (3¾ inches in diameter, water layer c. 2 inches thick), provided with windows of fused quartz. For better control of the lens-liver seal, the syringe-needle attachment was modified by the addition of a screw mechanism for moving the plunger, so that fluid could be delivered much more smoothly to the tip of the water immersion lens.

Respiration of rat liver slices in the presence of fluorescein, etc.: Male white rats (Carworth Farms), maintained on a diet of Purina Laboratory Chow and weighing 270 to 380 gm., were allowed food and water *ad lib.* until sacrificed by decapitation after being stunned by a blow upon the head. After the animals had bled freely from the neck vessels, the liver was promptly removed and placed in cracked ice. Thin slices were prepared with a pre-chilled Stadie-Riggs microtome (6), and 2 or 3 slices (total estimated weight, c. 200 mg.) were added to each experimental vessel. The experiments were carried out in conventional Warburg equipment, in a medium of Krebs-Ringer-phosphate (7), under the following conditions: reaction volume, 3.5 ml.; vessels kept in ice-water bath up to the time of gassing (2 minutes with frequent shaking); gas phase, pure oxygen; NaOH in center well; temperature, 37°; rate, 120 strokes per minute (3 cm. stroke); equilibration period, 5 minutes. All solutions of fluorescent compounds, prepared in Krebs-Ringer-phosphate (K-R-p), were placed in the side-bulbs of the vessels, and the requisite amount of K-R-p was added to the main compartment prior to the addition, immediately after preparation, of the liver slices. Each experiment was controlled by a thermobarometer and by a vessel containing, from the start, the full amount (3.3 ml.) of K-R-p in the main compartment. Fluorescent compounds were tipped into the main compartment 15 minutes after closure of the taps; and at the same time an equivalent volume of K-R-p was tipped into a control vessel.

Fluorescein (uranine) is freely soluble, and was tested up to a maximum concentration of 1:1,000. The solubility of thioflavine S and esculin in cold K-R-p is more limited; and it was deemed unwise to exceed, for addition to the side-bulb, a concentration of 1:500 for thioflavine S and of 1:1,000 for esculin. Under the circumstances, these two compounds were tested only to a maximum concentration of 1:5,000. In the case of esculin this necessitated the addition of 0.7 ml. of 1:1,000 solution to the side-bulb, and use of a separate tip-in control with an equivalent amount of K-R-p. Otherwise, all side-bulb additions were in a volume of 0.35 ml., the solutions of fluorescent compounds being diluted as required to give the desired final concentrations in the reaction mixtures.

Absorption and fluorescence emission spectra: Using the Hanovia Utility SH quartz mercury vapor lamp as a light source, fluorescence was excited (in silica cuvettes with 1 cm. light path) by a Farrand 300 mm. UV monochromator with 2 slit illuminators. The fluorescence spectrum was analyzed with an experimental Farrand grating monochromator (with a dispersion of 10 mμ per mm., slit 1 mm.), and recorded through a Farrand photometer equipped with RCA 1P21 phototube. The spectra were accomplished at the following concentrations: fluorescein and esculin, 1:100,000; thioflavine S, 1:50,000; proflavine, 1:200,000. The distilled water used for the solutions exhibited negligible fluorescence with the

wavelengths and the instrument sensitivity employed. The data have been recorded in terms of photocurrent output in strictly arbitrary units, which are a function of the particular equipment and experimental conditions. Accurate expression of the data in terms of relative intensity of fluorescence would be possible only if the spectral transmission characteristics of the analyzing grating monochromator, the spectral sensitivity of the phototube, and the relative incident fluxes for the different mercury lines were fully known. Such exacting and laborious calibration of the equipment has not been done, nor has any attempt been made to correct for self-absorption, since the data as recorded in Table I are entirely adequate for present purposes.

For comparison with the fluorescence emission spectra, ordinary absorption spectra were accomplished with the Beckman spectrophotometer (in silica cuvettes with 1 cm. light path), at the following concentrations: fluorescein, 1:100,000; esculin and thioflavine S, 1:50,000; proflavine, 1:150,000. The same samples of esculin and dyes (fluorescein, Coleman & Bell Co., lot no. 480347) which had been extensively employed in previous studies (2) were used for the fluorescence and absorption spectra. All solutions were freshly prepared in water which was first demineralized with conventional equipment, then distilled from alkaline permanganate in all-glass apparatus.

Radiation of liver with mercury vapor lamp: These experiments were all performed under standard conditions, employing the Hanovia Utility Quartz Mercury Vapor Lamp, provided with a new SH burner which was used for no other purpose until the experiments had been completed. The lamp was always well warmed up prior to use. Male specimens were used throughout; the frogs (*Rana pipiens*) were obtained from Vermont, the white mice from Carworth Farms (CF1 strain). Fluorescein was routinely administered as a 1:500 solution in balanced saline (Holtfreter's solution for frogs, mammalian Ringer's for mice) into the dorsal lymph sac of frogs and intraperitoneally in mice, in a dosage of 60 mg. per kg. Frogs were pithed 30 minutes and mice were sacrificed (by crushing of the cervical cord) 15 minutes after injection, and the liver was promptly exposed in the usual way. The distance between the tip of the SH burner (burned base up) and the surface of the liver was 8 cm. The Pyrex water cuvette used for these experiments was a standard Petroff culture flask (4372-A, A. H. Thomas catalog, 1950 ed.), with sidewalls c. 2 mm. thick, a water chamber 13 to 14 mm. thick, and a capacity of c. 100 ml. The quartz cuvette was provided with windows of fused quartz c. 1 mm. thick, with a water chamber c. 17 mm. thick, and a capacity of c. 25 ml. Both cuvettes were filled with boiled distilled water.

Exposures to radiation were accurately timed, and were of 2 minutes duration unless otherwise specified. The surface of the liver was irrigated with saline just prior to radiation and again at the end of 1 minute. The dome-shaped surface of the lobe exposed to the full force of the radiation was used for study, and was subjected to a more or less continuous, sweeping survey under the fluorescence microscope. Surveys were conducted routinely with the 55× water immersion lens and the 6× monocular. Unless otherwise specified, statements concerning the fluorescence intensity of the hepatic cells and of imbibition cells refer to the general cytoplasm, exclusive of the nucleus and any intracellular vacuoles of dye which may be present; and concerning bile canaliculi in the frog are meant to include both axial canaliculi and their blindly ending, intercellular branches.

The above statements apply equally to experiments involving cauterization of the liver surface. An electric cautery, with a V-shaped tip of rather fine wire, was used at red heat. The dosage of fluorescein in these experiments was 30 mg. per kg.

Comment upon vasoflavine: This dye was originally reported by Moses, Emery and Schlegel (8) as a purified fraction of thioflavine S, with almost ideal properties for visualization of blood vessels by Schlegel's (9) technic. We have used it extensively in studies of the mouse

liver (5), in which it gives pictures of bile canalicular delineation, etc. essentially equivalent to those obtained with thioflavine S.

OBSERVATIONS

We have derived most of our experience with the abnormalities here considered from animals injected with fluorescein in non-toxic dosage. Supplemental information has been obtained with the dyes thioflavine S and vasoflavine, as well as with the glycoside esculin. The abnormal phenomena are largely equivalent in both frog and mammal, irrespective of the fluorescent compound employed. However, the descriptions given below refer, unless otherwise specified, to the pictures observed in fluorescein preparations. The abnormalities have been encountered under a wide variety of experimental conditions, in pithed (frogs only), anesthetized and freshly sacrificed animals. The technic inevitably requires exposure of the liver, irrigation of its surface with saline, and more or less prolonged observation under the fluorescence microscope. None of these procedures is to be taken lightly, and the ultraviolet light in the incident beam of the microscope is well known to be potentially injurious to biological systems. Additional procedures employed include exposure of the liver to dry heat, radiation with visible and ultraviolet light of varying wavelengths, direct cauterization of the liver surface, etc. For illustrations of some aspects of the abnormal phenomena here considered, reference is made to the fluorescence microphotographs of Franke and Sylla (10, 11) and of Hanzon (12); and to the series of drawings published by Hirt, Ansorge and Markstahler (13). The more important figures from the latter work have been reproduced in color in a recent volume of this journal (2), and reference to these figures will make some of the following descriptions more readily comprehensible.

"Beading": The simplest abnormality which may develop in an otherwise normal preparation is what may be described as "beading" of the bile canaliculi. The phenomenon, which may develop in both frog and mammals (1, 2, 10, 11, 13), appears as a more or less irregularly discontinuous rather than a continuous delineation of the canaliculi by fluorescein, etc. In its earliest stage, the well delineated portions of the canaliculi appear entirely normal in caliber and otherwise. The intervening portions either are delineated at very low intensity, in which case they likewise appear normal in caliber; or, more usually, are not delineated at all, in which case one can obtain no valid information upon their true status. In more advanced "beading", the well delineated portions of the canaliculi may appear as more or less bulbous (oval or spherical) formations, of larger than normal caliber, not unlike a string of beads; and it was this simile that occurred to Hirt *et al.* (13).

"Tendril formation": This phenomenon, noted earlier in mammals sacrificed following the injection of thioflavine S (2), has been repeatedly encountered (in mice) in the course of the present study. It is characterized by the new de-

velopment of short, delicate, blindly ending branches from the canaliculi; and the observations would appear to leave no doubt as to its reality and distinctive nature.

"Sprouting": This expression aptly characterizes a phenomenon first described by Ansorge (14; see also 13, 15), and repeatedly confirmed in this laboratory in both frog and mammal (1, 2, 4). In its earliest form it consists of variably numerous, small, spherical protuberances or vacuoles, delineated by fluorescein, etc. at essentially the same intensity and color as the parent canaliculus, to which they are very closely applied. In this early stage it is usually impossible to determine whether the vacuoles are connected to the canaliculus by delicate channels, or represent simple outpocketings of the canalicular wall. In a more advanced stage many of the vacuoles, which may meanwhile have increased appreciably in size, are located at a variable distance from the canaliculus. It is then possible to observe quite clearly that at least some, and on occasion practically all of the vacuoles are connected with the canaliculus by delicate, dye-laden channels of varying caliber. Static pictures of "sprouting", at whatever stage of development, permit no valid conclusion as to its mode of origin. However, on occasion the entire phenomenon can be observed to develop under one's eyes from its very beginnings to its most flagrant form. When this occurs, the sequence of events is such as to leave no doubt that we are dealing with an actual "sprouting" of vacuoles from the canaliculi, rather than with secondary establishment of connections between the canaliculi and free intracellular vacuoles of the dye.

Intracellular vacuole formation: When well developed, the picture of intracellular vacuole formation is unmistakable, and is fundamentally the same in both frog and mammal. Spherical vacuoles containing fluorescein, etc.—varying in number, size and fluorescent intensity—are present within the cytoplasm of the hepatic cells. They may be more or less closely applied to the canaliculi, or may be separated by an appreciable, even considerable, distance from them or their loci. A variable and at times high degree of discontinuity of the associated canaliculi is the rule. As long as canalicular delineation persists, one can not infrequently observe quite clearly that some of the vacuoles are connected with the canaliculi by delicate channels, similarly delineated by the dye. The phenomenon may be exhibited by an isolated lamina or portion thereof, or even by a single hepatic cell, in an otherwise normal preparation. All intermediates between this and very widespread occurrence of the phenomenon may be observed under varying abnormal conditions.

Static pictures offer no information as to the mode of origin of the intracellular vacuoles, or of their connections (when such exist) with the canaliculi. When the phenomenon develops more or less rapidly in fields held under continuous observation, the sequence of events appears to agree in all essentials with that originally described by Hirt *et al.* (13). With the appearance and de-

velopment of "sprouting", the individual vacuoles may enlarge, coalesce and lose their connections with the canaliculi, which become more or less discontinuous and may disappear altogether. However, as noted elsewhere, in very numerous instances no connection between the vacuoles and the canaliculi can be visualized; "and some of the pictures obtained strongly suggest that, for many of the vacuoles at least, such a connection never existed" (2, p. 422).

In the mammal, as can be observed with particular clarity in the subcapsular laminae (2), intracellular vacuole formation frequently involves a polygon of the canalicular meshwork to give pictures quite different from those observed in the frog. It is not unusual to see a whole row of vacuoles lined up just inside the margins of a single hepatic cell territory, the surrounding cells being entirely free of them. On the other hand, one sometimes observes such a row of vacuoles just outside the limits of a given cell territory, itself not involved, and surrounding it in a way which can only mean selective, partial involvement of all of the adjacent cells.

Discontinuity of bile canaliculi: As stated above, intracellular vacuole formation is frequently accompanied by more or less extensive discontinuity of the associated bile canaliculi. The discontinuity is quite distinct from that observed in "beading", and of a far higher order of magnitude. Under some conditions (e.g., in fields held under continuous observation), such discontinuity may develop in the absence of intracellular vacuole formation.

Imbibition cells: We have adopted this term to designate hepatic cells which, in a given fluorescein or thioflavine S (vasoflavine) preparation of frog or mammal, exhibit—in comparison with the hepatic cells in general—a significantly higher level of cytoplasmic fluorescence attributable to the dye, and so stand out in more or less bold relief in the laminae involved. The term derives from the expression—"imbibierte Zellen"—used by Franke and Sylla (10, 11), who accurately described the phenomenon and interpreted the development of such cells as a manifestation of liver injury. The cytoplasmic fluorescence of imbibition cells may be of low, moderate or high intensity. They may occur singly or in groups of varying number. That they represent individual hepatic cell territories is immediately apparent in almost every instance. The territorial limits of isolated cells are precisely defined, the increased cytoplasmic fluorescence extending all the way to the cell barriers and contiguous canaliculi. However, particularly if their fluorescence is brilliant, it may be difficult or at times impossible to establish exact cellular limits at points where two affected cells abut upon each other, and share a common barrier or canaliculus which is delineated at approximately the same intensity.

In our experience, the nuclei appear as a negative image or cannot be visualized at all. At varying levels, including the highest, of fluorescent intensity of the cytoplasm, the nuclei may be more or less sharply delineated as spherical, dark, entirely or relatively non-fluorescent areas, devoid of structural detail.

Many of the pictures are quite striking, and suggest that the nuclei have been completely spared by the dye. We cannot recall ever having observed an imbibition cell in which the nucleus was delineated by the dye at a fluorescent intensity higher than that of the cytoplasm. Occasionally in the frog, more frequently in the mammal, from one to numerous vacuoles, delineated by the dye at appreciably higher fluorescent intensity than that of the general cytoplasm, may be present in otherwise typical imbibition cells. The vacuoles are routinely peripheral in location, and applied more or less closely to the canaliculi. We have as yet been unable to visualize any channels connecting the one with the other. However, these could obviously be present and completely obscured by the fluorescence of the cytoplasm.

In the frog, when selected microscopic fields are held under continuous observation, imbibition cells may develop at highly variable rates of speed. At one extreme is the slow emergence of one or more isolated cells from the general background, requiring up to several minutes to attain high fluorescent intensity; at the other extreme is an almost explosive development. Except in the latter case, when accurate analysis is impossible, the increase in cytoplasmic fluorescence appears always to be a smooth, continuous process, with no wave of spread across the cell, and no suggestion that the dye is being received from one or more specific points at the periphery. There is no doubt that an hepatic cell, already the site of intracellular vacuole formation, may develop into an imbibition cell, as described by Hirt *et al.* (13). However, in our experience, preexisting or simultaneous intracellular vacuole formation is neither a necessary nor a typical feature of imbibition cell development. In fields held under continuous observation, just as imbibition cells may develop under one's eyes, they may likewise gradually regress, their greater cytoplasmic fluorescence diminishing until they can no longer be distinguished from their neighbors.

Imbibition cells would appear to have two possible fates: the one is death, the other recovery. Ultimate cell death we have observed repeatedly. Of apparently complete recovery within a reasonable time, we have seen relatively few examples; however, some of our observations would seem to admit of no other interpretation.

Earlier reference has been made (1, 2) to so-called "lakes" of fluorescein, the expression being derived from Hirt *et al.* (13–15), who described "Fluoresceinseen" in the later stages of biliary excretion. It is obvious that at least most of Hirt *et al.*'s "lakes" were nothing more than imbibition cells, occurring singly or in groups. Whether any of them constituted a distinct phenomenon is doubtful, but cannot be excluded.

"*Turbidity*": Under conditions of severe injury (e.g., prolonged observation under the microscope, exposure to heat and ultraviolet radiation, and direct cauterization of the liver), the laminae frequently develop what may be called a "turbid" appearance, with generalized staining of the hepatic cells, giving

dull, uninformative pictures as compared with the usual luminous, fluorescent images. In our experience, the development of "turbidity" is a sign of cell death.

Respiration of rat liver slices in the presence of varying concentrations of fluorescein, thioflavine S and esculin

In studies of this sort, the fluorescent compounds are routinely employed in a dosage well below toxic levels. However, in view of the development of such manifold abnormalities, it seemed highly desirable to obtain additional information concerning possible direct effects of these compounds upon hepatic function. For this reason, experiments were performed upon the respiration of rat liver slices in the presence of varying concentrations of fluorescein (1:100,000 to 1:1,000), thioflavine S and esculin (1:100,000 to 1:5,000 in each case). The data obtained would appear to justify the conclusion that fluorescein, thioflavine S and esculin—over the range of concentration tested and under the experimental conditions employed—have no significant effect upon the endogenous respiration of rat liver slices.

Monochromatically excited fluorescence emission spectra of fluorescein, esculin, thioflavine S and proflavine

Although they may appear in the absence of significant exposure to the incident beam of the microscope, many of the abnormalities described above develop repeatedly in livers subjected to more or less prolonged observation. Under the circumstances, we were particularly interested in studying the development of such abnormalities in livers directly subjected to radiation by both visible and ultraviolet light of different wavelengths. The usual practice in fluorescence microscopy is to use, as a light source, the carbon arc or the mercury vapor lamp; and to bombard the organs or tissues with a beam from which visible light has been removed more or less completely by suitable filters. In recent years the mercury vapor lamp has largely supplanted the carbon arc, and the beam employed sometimes includes visible blue light of considerable intensity. In the mercury spectrum, it is the intense line at 436 mμ which is primarily responsible for the blue component when such a beam is employed. In our own equipment, this line is almost entirely excluded from the beam, but the doublet at c. 405 mμ is well transmitted. In the ultraviolet region, with the filters conventionally used, the intense mercury line at 365 mμ is of principal importance in exciting the fluorescent images obtained.

As a basis for more intelligent investigation of radiation effects, we thought it best to obtain first-hand acquaintance with the intensity and spectral character of the fluorescence of fluorescein, as excited monochromatically by the 365 and 436 mμ lines of the mercury vapor lamp. As a background for further

studies of the liver and other organs, such fluorescence emission spectra were accomplished not only with fluorescein, but also with thioflavine S, esculin and proflavine. The latter compound is representative of the basic fluorescent dyes,

TABLE I

Monochromatically excited fluorescence emission spectra of esculin, thioflavine S, fluorescein and proflavine

WAVELENGTH (millimicrons)	FLUORESCENCE—PHOTOCURRENT OUTPUT IN ARBITRARY UNITS				
	Excited by Hg 365 mμ			Excited by Hg 436 mμ	
	Esculin 1:100,000	Thioflavine S 1:50,000	Fluorescein 1:100,000	Fluorescein 1:100,000	Proflavine 1:200,000
380	4.5	4.8	—	—	—
390	6.0	6.3	—	—	—
400	8.8	10.0	0.5	—	—
410	13.0	20.5	—	—	—
420	24.2	40.0	0.8	—	—
430	48.0	66.5	—	—	—
440	74.5	82.5	1.0	—	—
450	94.0	89.5	—	3.0	4.5
460	102.0	91.0	1.3	4.2	9.0
470	100.3	83.0	2.0	6.5	19.0
480	92.0	70.2	4.0	15.0	38.0
490	79.0	56.6	11.0	34.0	59.5
500	67.0	42.0	22.0	67.5	76.0
505	—	—	29.2	85.5	82.0
507.5	—	—	30.9	89.9	—
510	54.0	32.6	33.0	96.0	84.2
512.5	—	—	34.0	97.8	—
515	—	—	35.2	97.8	86.0
517.5	—	—	35.2	98.8	—
520	42.5	23.8	34.7	97.5	84.8
525	—	—	33.3	96.0	80.0
530	31.0	17.2	30.8	90.0	75.5
540	23.6	13.0	27.0	80.7	69.5
550	16.5	—	23.3	70.2	58.0
560	13.0	8.0	19.0	58.0	45.0
570	—	—	14.5	44.5	35.4
580	7.0	5.7	10.5	33.0	26.0
590	—	—	7.8	23.5	20.0
600	4.0	4.0	5.0	15.7	14.0
620	—	—	2.0	5.5	5.5
640	—	—	1.1	3.0	3.0
650	2.0	0.4	—	—	—
660	—	—	0.9	2.0	2.0
700	2.0	0.4	0.8	1.0	1.3

which are highly useful for visualization of some aspects of the hepatic cell cytoplasm and nuclei, though not of the bile canaliculi. The principal results of this study are presented in Table I and in the accompanying consolidated chart

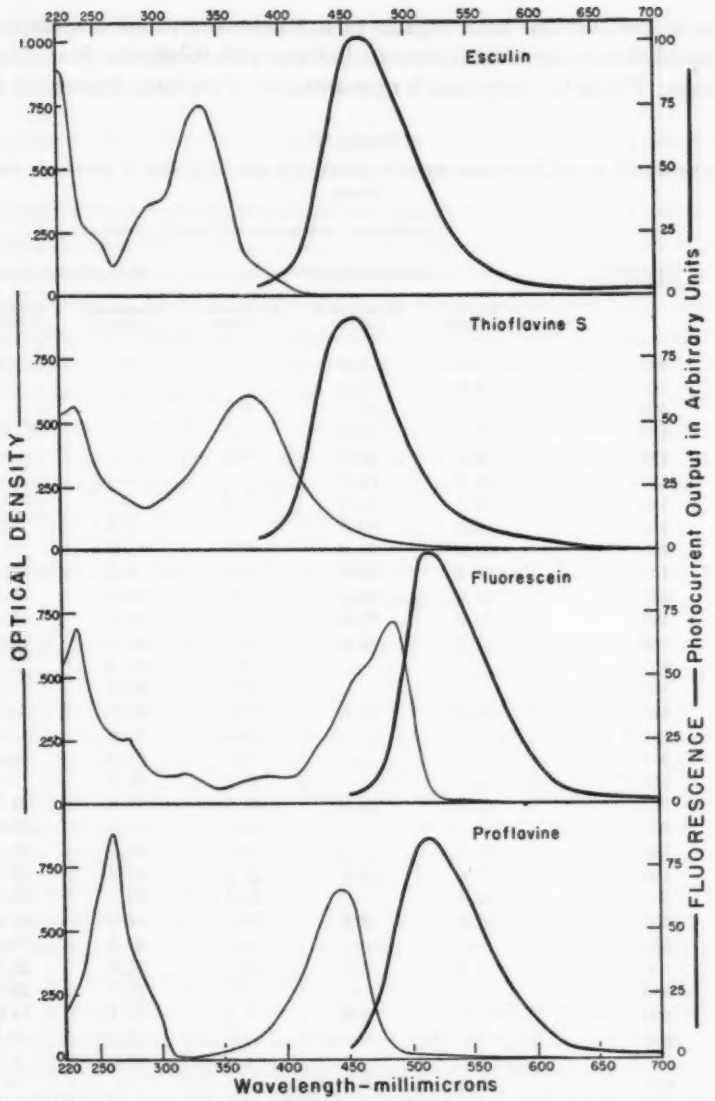

FIG. 1. Absorption and monochromatically excited fluorescence emission spectra of esculin, thioflavine S, fluorescein and proflavine. Fluorescence spectra of esculin and thioflavine S excited by Hg 365 mμ; of fluorescein and proflavine, by Hg 436 mμ.

(Fig. 1). Esculin and thioflavine S, which characteristically exhibit a blue fluorescence, were excited to intense fluorescence by Hg 365 mμ, but did not respond at all to Hg 436 mμ under the experimental conditions employed. In

analyzing the fluorescence spectra of fluorescein, as excited by Hg 365 mμ and Hg 436 mμ, the recording instrument was set at the same sensitivity. It would appear that both of the Hg lines excite fluorescence of essentially the same spectral character. A similar situation prevails with proflavine, but in this case the data for Hg 365 mμ are not reported in detail, since the low intensity of the fluorescence required a marked increase in the sensitivity of the recording instrument. With the latter deliberately set to read 100 at 520 mμ, with excitation by Hg 436 mμ, the following readings were obtained: excitation by Hg 365 mμ, 14.0; excitation by Hg 405 mμ, 30.0.

For those interested in utilizing these compounds in fluorescence microscopy, with the mercury vapor lamp as a light source, there are certain obvious lessons to be learned from this study. With esculin and thioflavine S, while there is no escape from working with ultraviolet light, this can be limited to the region of Hg 365 mμ. With fluorescein and proflavine, on the other hand, while both are excited to fluorescence by Hg 365 mμ, ultraviolet light can—if it is so desired—be completely excluded from the incident beam, which can be limited to the region of Hg 436 mμ. Since both of the mercury lines excite fluorescence of essentially the same spectral character, a comparison of the effects obtained with Hg 365 mμ and Hg 436 mμ—when used separately—should prove particularly helpful in the analysis of various aspects of radiation effects, photosensitivity and photodynamic action, in so far as these modify or distort normal vital processes.

Development of abnormalities following radiation of exposed liver surface by quartz mercury vapor lamp under varying conditions

Observations upon the frog liver: These radiation experiments were carried out primarily *in vivo* upon the liver of the pithed frog, in which the complicating factors of anesthesia and temperature control could be avoided. In such preparations, without any additional radiation over and above that provided by prolonged observation under the microscope, a wide range of abnormalities—variable in type and degree—may develop with time, and these must be discounted in evaluating effects attributable to a given experimental procedure.

In exploratory experiments with selective filters, it became obvious that radiation of the liver surface with wavelengths above 400 mμ—ultraviolet light being completely excluded—did not lead to the development of abnormalities significantly different in type, degree or time relationships from those observed in control animals. We were particularly interested to note the absence of appreciable effects from radiation with the 436 mμ line of the mercury spectrum, in view of its efficiency in exciting the characteristic fluorescence of fluorescein. We then extended the radiation to include ultraviolet light down to the region of c. 300 mμ, by removing all color filters and interposing between the mercury lamp and the liver only a water cuvette provided with Pyrex windows. Under these conditions, the liver developed with time significantly

more abnormalities than the control animals; but these were largely confined to "beading" and "sprouting", with only occasional instances of intracellular vacuole formation and imbibition cells. However, one of the frogs developed during the initial survey period a rather considerable number of imbibition cells, many of which exhibited apparently complete recovery during the ensuing hour or so.

The radiation was again extended to include ultraviolet light of wavelengths below 300 mμ by interposing between the mercury lamp and the liver a water cuvette provided with quartz windows. In comparison with the preceding, these preparations were very striking indeed. At the very beginning of observations, the pictures—with few exceptions—were essentially normal in all particulars. Then, under one's eyes, the liver rapidly developed into a virtual museum of abnormalities, highly variable in type and degree; and it became obvious, with time, that many of the hepatic cells had become so severely injured as to result in their death within a relatively brief period. The findings are illustrated by the following protocol:

Frog A31, 24.4 gm.:

0: Injection of 0.73 ml. of fluorescein 1:500. *30′:* Brain and cord pithed, liver exposed. *32′35″–34′35″:* Radiation of liver.

35′0″–45′0″: Survey of left upper lobe, ventral surface. Initially, the pictures are essentially normal. The bile canaliculi are sharply delineated at high intensity, and the dye is detectable in all hepatic cells at between low and moderate intensity. Circulatory activity is readily apparent in numerous sinusoids and all larger vessels. *37′0″:* First imbibition cells noted (several in 1 field, moderate or high intensity). *37′30″:* A field showing 2 imbibition cells (high) and 2 unequivocal instances of delicate "sprouting". *38′0″:* A field showing widespread "sprouting" and intracellular vacuole formation, and numerous imbibition cells. *38′25″:* Another field like the preceding. *38′50″:* An area of considerable extent with delicate "sprouting" in almost all laminae. *39′10″:* First imbibition cell noted in above area. *39′40″:* The preparation is now characterized in general by widespread "sprouting" (degree variable), very numerous imbibition cells (intensity quite variable, at times very high), and suggestive "turbidity" of the hepatic cells in many laminae (particularly in areas showing numerous imbibition cells). *40′20″:* Almost every field now shows abnormalities, always "sprouting", usually 1 or more laminae with intracellular vacuole formation, and frequently from 1 to numerous imbibition cells. *43′0″:* In a survey beginning at this time, the 2 most outstanding features of the preparation are widespread "sprouting" (all degrees) and the presence of numerous imbibition cells (regional variation, intensity highly variable). Less prominent features are intracellular vacuole formation (very numerous instances) and "turbidity" of the hepatic cells (most readily apparent in fields with most numerous imbibition cells). There are numerous instances of more or less delicate "beading". A small minority of the canaliculi are still entirely devoid of detectable abnormality. In general, the hepatic cells still exhibit fluorescein in their cytoplasm, at very low, low or somewhat higher intensity.

55′0″–61′0″: Resurvey of left upper lobe, ventral surface. The preparation now shows widespread "sprouting", intracellular vacuole formation with frequent discontinuity of the associated canaliculi, and imbibition cells. Intracellular vacuole formation, of all degrees, is so frequent and striking that it has now superseded "sprouting" as a principal feature of the

preparation. Practically every field shows several or more imbibition cells (usually moderate or high, frequently very high, sometimes low intensity). In hepatic cells not so affected, "turbidity"—with fluorescein staining at low intensity—is frequently apparent. In general, the dye is still detectable in the hepatic cells (very low or low intensity, sometimes higher). Essentially normal canaliculi, with or without delicate "beading", are still found in occasional laminae. Circulatory activity was not detected, but no particular effort was made to do so.

75'0"–81'0": Resurvey of left upper lobe, ventral surface. Every field now exhibits gross abnormality, with varying numbers of imbibition cells, several or more laminae showing predominantly intracellular vacuole formation, and other laminae with varying degrees of "sprouting". In some fields the very numerous imbibition cells give a patchwork appearance, the remaining hepatic cells being "turbid" and stained with fluorescein at low intensity. In contrast to earlier findings, many imbibition cells are now rather irregular in outline, sometimes almost leaf-like in contour, as if shrunken. Intracellular vacuoles are usually of medium or large size, sometimes very large (but not of cellular size), and always spherical. The majority of the vacuoles are of high or very high intensity, but many (particularly those of medium size) are of only moderate or even low intensity. It is noteworthy that numerous laminae, occurring singly or in small groups, are now devoid of detectable fluorescein except in the general cytoplasm of the hepatic cells (very low or low intensity). Only rare isolated laminae still show essentially normal canaliculi, and even these frequently exhibit "beading". Circulatory activity can be readily visualized in some of the larger vessels, but not in the sinusoids. Fluorescein is apparently still detectable in the plasma of active vessels, at very low or low intensity and greenish in color.

105'30"–110'30": Resurvey of left upper lobe, ventral surface. Compared with the preceding period, there is a significant reduction in intracellular vacuole formation, and many more of the vacuoles are now of low or moderate intensity. Also, many more laminae (in some areas, roughly half) now show no detectable trace of fluorescein except for general cytoplasmic staining at low intensity. The number of imbibition cells has apparently increased, and those of irregular contour (low or moderate intensity) now decidedly outnumber typical imbibition cells of regular contour. Many scattered instances of "sprouting" (degree variable) are still present. Essentially normal canaliculi, with or without "beading", are very rare but still persist. A "turbid" appearance of the hepatic cells, with fluorescein staining of the cytoplasm at low intensity, is rather widespread. Circulatory activity is readily apparent in some of the larger vessels.

130'0"–136'0": Survey of left upper lobe, *dorsal* surface. The pictures are everywhere surprisingly normal. Fluorescein is detectable in all hepatic cells, usually at moderate intensity. The canaliculi are richly delineated at high intensity, and with few exceptions are entirely normal in appearance. Occasional instances of delicate "sprouting" and a single lamina showing intracellular vacuole formation were encountered in a wide survey; there were no imbibition cells. Circulatory activity was detectable in the larger vessels, but not in the sinusoids.

In a final series of experiments, the water cuvette was removed and the liver was subjected to radiation by the entire range of the mercury spectrum, with its accompanying heat. The sequence of events was fundamentally the same as before except for time relationships. Approximately equivalent degrees of injury and abnormality were obtained more quickly with unshielded radiation than when the quartz cuvette was interposed.

Observations upon the mouse liver: A few exploratory experiments were per-

formed upon mice sacrificed at the peak of excretion of fluorescein, with radiation of the liver under conditions equivalent to those used in the frog (except that trials with selected portions of the visible spectrum were omitted). The critical difference is that these mouse experiments were performed not *in vivo*, but after death; and one would hardly anticipate comparable results. Nevertheless, the findings were surprisingly similar as to both type and degree of abnormalities under the varying conditions.

Development of abnormalities following direct cauterization of liver surface

Following direct cauterization of the liver at the peak of fluorescein excretion, in the pithed frog and the freshly sacrificed mouse, a wide variety of abnormalities—largely of the same types noted in radiation experiments—appear promptly and develop progressively in the zones peripheral to the burned areas. The following protocol is self-explanatory. Similar pictures, though of lesser degree, may be produced in livers subjected to the heat of the cautery, without direct contact.

Frog A3, 21.4 gm.:

0: Injection of 0.32 ml. of fluorescein 1:500. *30':* Brain and cord pithed, liver exposed. Ventral surface of left upper lobe cauterized in 5 separate areas.

33'25"–47'50": Survey of left upper lobe, ventral surface. In the most severely burned areas hepatic structure has been largely destroyed; the laminae are recognizable with difficulty at best, and are apparently not stained with fluorescein. In less severely burned areas the laminae are clearly defined and "turbid" in appearance, with fluorescein staining at from low to moderate intensity. Adjacent to the obviously burned areas the liver exhibits the most extraordinary display of intracellular vacuole formation, with widespread discontinuity of the bile canaliculi. Many of the vacuoles are of medium and large size, and all are delineated by fluorescein at high intensity. Some of the vacuoles are clearly connected to the canaliculi by delicate channels, but the majority are apparently devoid of such connections. More peripheral to the burned areas the pictures become progressively more delicate, and are characterized predominantly by "sprouting". Still more peripherally, the pictures of bile canalicular delineation, on the whole at moderate intensity, are entirely normal. Fluorescein is not detectable in the hepatic cells or in the plasma.

81'15"–83'0": Resurvey of left upper lobe, ventral surface. The areas previously showing marked intracellular vacuole formation now exhibit numerous imbibition cells, singly or in small groups and displayed with great clarity. Their cytoplasm is of high intensity, and their nuclei are frequently delineated as non-fluorescent areas.

DISCUSSION

This discussion will be largely confined to a presentation and evaluation of the observations of earlier and contemporary investigators by the technic of fluorescence microscopy, in so far as they bear upon the abnormal phenomena here considered. No attempt will be made to review the extensive literature upon abnormalities of the liver as studied by other methods, or upon the character of the absorption and fluorescence emission spectra of the various com-

pounds employed. Our own spectra are in no sense unique, but have provided information which was useful to us, and are reported here in the belief that they may prove of interest to others.

In their pioneering investigations of living organs *in situ*, Ellinger and Hirt (16, 17) studied the excretion of fluorescein by the frog's liver; and they make no mention of having noted any abnormalities except for the development of diffuse staining in a particular field when studied at the highest magnification. In their studies of the frog's liver under varying conditions (including X-radiation), Hartoch and Israelski (18–20) appear to have encountered only two of the abnormalities here discussed, "beading" and discontinuity of the canaliculi.

Franke and Sylla (10) investigated the excretion of fluorescein by the frog's liver under normal and abnormal conditions (prolonged observation under the fluorescence microscope, biliary obstruction and the effect of various poisons). They observed, and recognized as abnormal, the phenomena of "beading", discontinuity of the canaliculi, intracellular vacuole formation and imbibition cells; and their figure 8 shows sparing of imbibition cell nuclei by fluorescein. They also observed such cells in frogs injected with eosin, and noted that in this case the nucleus exhibited a reddish fluorescence, the cytoplasm being bright yellow. In phenylhydrazine poisoning, with the development of numerous imbibition cells, they were unable to recognize any concomitant pathological changes in ordinary histological preparations; and concluded that the phenomenon of dye imbibition can probably be regarded as the earliest demonstrable expression of hepatic cell injury. They included in their report a single photograph of fluorescein excretion by the rat's liver, and this clearly shows the phenomena of "beading", intracellular vacuole formation and imbibition cells. They subsequently (11) accomplished a similar study of fluorescein excretion in mammals, and again observed intracellular vacuole formation and imbibition cells. The latter appeared in normal preparations subjected to prolonged observation and, as before, were interpreted as an early sign of cell injury, since their presence could not be detected in routine sections. Many of Franke and Sylla's observations are presented again in two later articles by Franke (21, 22). He notes (21) that in phenylhydrazine poisoning imbibition cells can appear in large numbers prior to delineation of the canaliculi; and regards it as unlikely that they are to be explained on the basis of subsequent re-entry into the hepatic cells of dye which had first been concentrated in the canaliculi. He is inclined to assume that imbibition cells have lost their ability to transfer fluorescein to the canaliculi, but can probably still transfer it to the blood and lymph.

Quite independently of Franke and Sylla, Ansorge (14) at the same time studied the excretion of fluorescein (and trypaflavine) by the liver of the winter frog. Although she did not appreciate their abnormal character, she repeatedly observed "beading", "sprouting" (which she was the first to describe), intra-

cellular vacuole formation, discontinuity of the canaliculi and imbibition cells. The latter she designated as "Seen" or "lakes" of fluorescein; and in a description of postmortem changes indicated that the nuclei were spared by the dye. In biliary obstruction, she observed simultaneous development of imbibition cells and loss of fluorescein from adjacent canaliculi; and further noted that the dye could subsequently be released from imbibition cells into the sinusoids. We have yet to observe a single instance of the latter phenomenon. Markstahler (15) performed a similar series of experiments in the white rat. Again without appreciating their aberrant character, she encountered the same varieties of abnormal phenomena. However, although she assumed its occurrence, she did not actually visualize release of fluorescein (in biliary obstruction) from imbibition cells into the sinusoids. The subsequent report of Hirt, Ansorge and Markstahler (13) is essentially a repetition of the theses of Ansorge (14) and Markstahler (15), supplemented by a series of drawings by the distinguished artist, August Vierling.

Roller and Schober (23), in allyl formate poisoning in the salamander, noted on occasion the development of imbibition cells in laminae immediately adjacent to larger vessels. In fluorescein studies of salamander larvae, Sturm (24, 25) observed in two animals unusual pictures suggesting intracellular vacuole formation.

Hanzon's recent monograph (12) reports a detailed analysis of biliary excretion in the rat, under normal and abnormal conditions; and his *in vivo* observations are supplemented by descriptions of the fluorescent images obtained with fluorescein, as preserved in frozen-dried material. He is the first to accomplish studies of the latter sort, and they afford much interesting information. His monograph is documented by a large and excellent series of fluorescence microphotographs. Discussion of his work will be limited to those features which have a direct bearing upon our present and earlier procedures and findings.

In discussing his improvements in technic, Hanzon emphasizes the importance of markedly increasing the intensity of his incident beam, and implies that the resulting greater brilliance of the fluorescent images permits greater accuracy of observation. We seriously doubt that this is the case, and regard such increased intensities as not only undesirable but highly objectionable in the study of living or freshly sacrificed animals. His description of the canalicular system in the rat is fairly in accord with our earlier (2) and subsequent experience, except for the inclusion of "tuft-like formations with dead ends" as a feature of its normal architecture. This we would deny. His description of these tufts would fit some variants of our "tendril formation". Hanzon emphasizes the advantages of photography of the fluorescent images, and vigorously denies (p. 76) the occurrence, under any conditions, of channels connecting intracellular vacuoles with canaliculi. To this we must take equally vigorous

exception. Also, we are unwilling to concede the superiority of photography over the capacity of the human eye in studies of this sort.

The phenomenon of "sprouting" reveals an extraordinary plasticity of the bile canaliculi under abnormal conditions. Also, the rapidity with which "tendril formation" and "sprouting" have on occasion been seen to develop, in an initially normal preparation, almost certainly offers at least a partial explanation for some of the claims to have demonstrated intracellular branches of the canalicular system as a feature of its architecture under normal conditions. The recent findings of Wachstein and Zak (26) in the rabbit, with the alkaline phosphatase technic, could be readily explained on this basis. Himsworth (27, p. 128), in his text, describes the canaliculi as "ordinarily no more than potential spaces between the parenchymal cells (Fig. 46)." However, in the legend to the figure cited he refers to the "canaliculi which lie between the parenchymal cells and penetrate into their substance." The figure itself is most interesting. It purports to show the canaliculi in the normal rat, as demonstrated by retrograde injection of the bile ducts with India ink. In terms of our experience, the canaliculi are for the most part grossly abnormal, and quite clearly exhibit varying degrees of "sprouting".

Hanzon reports experiments upon graded injury to the liver by ultraviolet radiation (350–400 mμ) through the microscope objectives, and notes the sequence of regression of canalicular delineation, development of imbibition cells, and diffuse staining of the laminae. However, he makes no mention of "sprouting" or of intracellular vacuole formation, which form variably prominent features of our preparations; and we have never observed under any conditions the swelling of hepatic cells which he describes and illustrates. He found that imbibition cells were more surely produced when radiation was coupled with biliary obstruction; and he implies transfer of fluorescein from canaliculi to imbibition cells. Furthermore, under these conditions, fluorescein was often observed to flow from imbibition cells into adjacent sinusoids; this we have never seen. His frequent finding, in imbibition cells, of nuclear fluorescence stronger than that of the cytoplasm is contrary to our experience. He likewise noted development of imbibition cells under a variety of other conditions, and found that they may exhibit no detectable abnormality when stained with iron hematoxylin in frozen-dried material; this is in accord with the experience of Franke and Sylla (10, 11). Faced with its development under widely different conditions, Hanzon concludes (p. 193) that the imbibition cell "seems to be a general reaction of the cells to injury." Many of his observations are consistent with the idea that imbibition cells may return to normal within a reasonably short time, and with this we would agree.

Himsworth (27) emphasizes the predominant susceptibility to injury of the hepatic parenchymal cells, in comparison with those of the associated biliary

and reticulo-endothelial systems (Kupffer cells). Throughout his monograph there runs the recurrent theme of "the category of automatic, non-specific reactions of the liver to injury"; of the lack of characteristic responses to specific causes, but "simply the normal reaction of the liver to injury whatever the cause of that injury" (p. 14). Granted that Himsworth is speaking for the most part of grosser aspects of hepatic disease and injury, his views hold much that seems pertinent to the problems that concern us here. Over a wide range, whatever we do to the liver brings in its train, by and large, the same varieties of abnormality. Earlier studies led us to believe that, if any single factor plays an essential r le in the development of these abnormal phenomena, anoxia seemed the most likely possibility. On the basis of our more recent experience, we would question the validity of this conclusion.

SUMMARY

Fluorescein (in the form of its water-soluble sodium salt, uranine) and thioflavine S (in its conventional or more purified form, vasoflavine), as well as the glycoside esculin, have been extensively employed in studies of the mechanism of biliary excretion, in amphibia and mammals, by the technic of fluorescence microscopy. Under varying conditions (including more or less prolonged observation under the fluorescence microscope, ultraviolet radiation, anesthesia, anoxia, etc.), there may develop a wide variety of abnormal phenomena, which are in general highly repetitive in character. These abnormalities include the following: "*beading*"—a more or less irregularly discontinuous rather than a continuous delineation of the bile canaliculi; "*tendril formation*"—the new development of short, delicate, blindly ending branches from the canaliculi; "*sprouting*"—literally a sprouting of numerous, small vacuoles from the canaliculi, with which many of the vacuoles remain connected for a time by delicate, dye-laden channels of varying caliber; *intracellular vacuole formation*—the appearance within the cytoplasm of the hepatic cells of spherical vacuoles, varying in number, size and fluorescent intensity, and arising either as a sequel to "sprouting" or at times, so far as one can tell, independently thereof; *discontinuity of bile canaliculi*—a discontinuity which is more extensive than that observed in "beading", and is a frequent accompaniment of intracellular vacuole formation, but may develop in its absence; *imbibition cells*—hepatic cells which exhibit, in comparison with the hepatic cells in general, a significantly higher level of cytoplasmic fluorescence attributable to the dye, and so stand out in more or less bold relief in the laminae involved; "*turbidity*"—the development of a "turbid" appearance of the laminae, with generalized staining of the hepatic cells, and apparently a sign of cell death.

General descriptions of each of these distinctive phenomena are supplemented by observations upon the phenomena as they develop following radiation of the liver with ultraviolet light, and following cauterization of its surface.

Taken as a whole, these abnormalities constitute a most revealing picture of the distortion of normal hepatic function under varying experimental conditions.

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MALDEVELOPMENT OF THE CORIUM IN THE OSTEOGENESIS IMPERFECTA SYNDROME

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We have recently called attention to certain hitherto unreported morphological alterations in the skin, eye, and skeletal tissues of an infant coming to autopsy with the classical manifestations of osteogenesis imperfecta congenita (1). In the skin, the primary change appeared to be a failure of the normal transformation of reticulum fibrils into mature, non-argyrophilic collagen fibers. Since this abnormality had not been described before, dermal tissues of subsequent cases of osteogenesis imperfecta have been examined with great interest. It is the purpose of this report to present observations on the skin from 4 additional cases which have become available for study since the publication of our previous communication (1).

CASE REPORTS

Case 1 (23978). A colored male infant (1180 gm.) was born of a normally developed mother following premature rupture of the membranes. Pregnancy was estimated as 32 weeks in duration. Maldevelopment of the extremities was noted; X-ray examination revealed multiple fractures. After 15 days in a respirator unit during which time there was no weight gain, the infant suddenly stopped breathing. Stimulants failed to restore respirations or pulse.

At autopsy, the gross appearance was typical of osteogenesis imperfecta: pseudo-micromelic tapering and angulation of the extremities and a soft skull. Sclerae were slightly blue. Liver weighed 56 grams; spleen weighed 5.5 grams.

Microscopically, the skeletal tissues showed characteristic changes of osteogenesis imperfecta (1). Histochemical observations on the bones will be detailed elsewhere (2).

Sections of the skin showed a prominent deviation from the normal (Figs. 1–4). While the epithelium was of usual thickness, the corium was not. A striking difference with the normal control from an infant of the same weight was the way in which the hair follicles extended through the thin corium into the underlying fat. The follicles in the normal were, as usual, contained within the collagenous fibers of the corium. The great decrease in width of the latter was further accentuated by the fineness of all of the fibers. The delicacy of these fibers was brought out in the hematoxylin and eosin preparations (Figs. 1 and 2). However, silver impregnation technique Gomori (3) revealed their argyrophilic nature (Figs. 3 and 4), thus classing them as reticulum. Staining with the periodic acid-Schiff technique (PAS) (4) further characterized them as reticulum. Fibroblasts were present in the corium but did not appear decreased in numbers over the controls.

Because of certain observations which have been carried out in this laboratory on the

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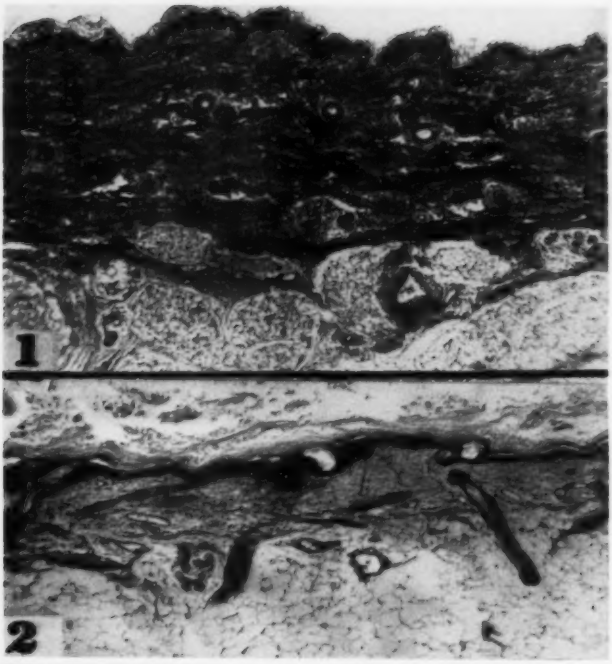


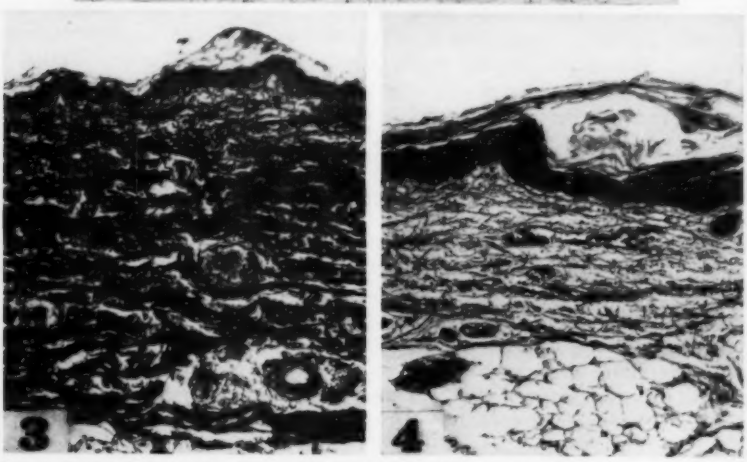


FIG. 1. Control, H and E stain, from newborn infant weighing 1250 grams. Note thickness of corium and presence of hair follicles within layer of connective tissue fibers.

FIG. 2. Section, H and E stain, same magnification as Figure 1, from Case 1. Compare width of epithelial layers. They are the same. Note thin and delicate fibered corium. Note how hair follicles project through this structure into fat.

FIG. 3. Control, Gomori (3) silver stain, skin. The dark staining collagen fibers are not black (argyrophilic) but rather have a purplish color.

FIG. 4. Section, Gomori (3) silver stain, from Case 1, same magnification as Figure 3. Note delicacy of fibrils which are black (argyrophilic).

shrinkage temperature of skin and cartilage (5), this physical property of segments of skin from this autopsy was investigated. Thin strips of corium were gradually heated in water, beginning at temperatures of 40°C. and continuing up to 85°–90°C. One end of strip was fixed while the opposite end was fastened to a writing lever which was placed in contact with a revolving smoked drum. Temperature readings were made every 2 minutes. The result of this experiment is shown in Figure 5 where it will be noted that sudden contraction occurred at about 66°–67°C., which is entirely normal. The significance of this will be discussed below.

Case 2 (*A-22803*). Abnormalities of the skin in this case, including changes in the bones and eyes, have been described elsewhere (1). In brief, this was a negro infant, weighing 1400 grams, who was delivered following a prolonged labor. Parents were normal, as were 3 siblings. At autopsy, the skin was noted to be thin.

Microscopically, the epidermis appeared entirely normal when compared with that from an infant of the same birth weight. In the hematoxylin-eosin sections, the fibers of the corium appeared thinner than those of the normal controls. Reticulum stains (3) revealed

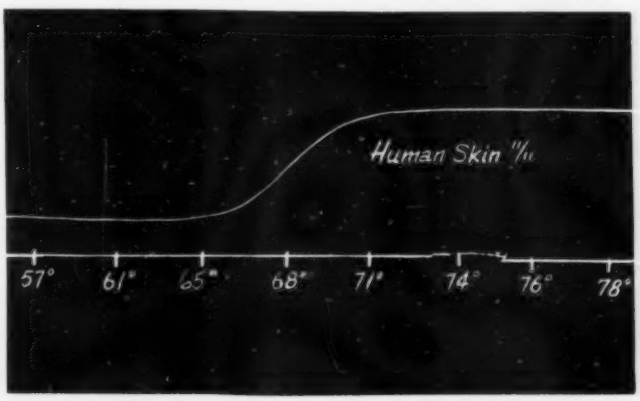


FIG. 5. Shrinkage temperature tracing of strip of skin from Case 1. Note contraction begins at about 65°–66°C. This is the usual temperature of contraction for normal human skin collagen.

the true nature of the fibers in the corium; they were argyrophilic (Figs. 6 and 7). Virtually no non-silver staining fibers were present; in other words, no fibers corresponding to the definition of "collagen" were present. In addition to the positive silver staining reaction, the fibers exhibited a red color with the periodic acid-Schiff reagents (4).

Case 3 (*23735*). A 2 year old white female was born with a fracture of the right femur. There had been 20 to 25 post-natal fractures of various bones. Sclerotics were pale blue. She was admitted to the Children's Hospital School for the purpose of an osteotomy and intramedullary nailing of the left femur. She withstood the procedure well but an hour later went into shock and despite transfusion and other therapy, respirations ceased.

At autopsy, aside from the skeletal deformities no gross abnormalities were found. Microscopically the skeletal tissue available for study (rib) was normal. Sections of the skin showed normal covering epithelium. The corium (Fig. 9) when compared with the normal (Fig. 8) of the same age was somewhat thinner. However, were one not on the lookout for some change the sections of the skin would doubtless be passed over as normal. Silver

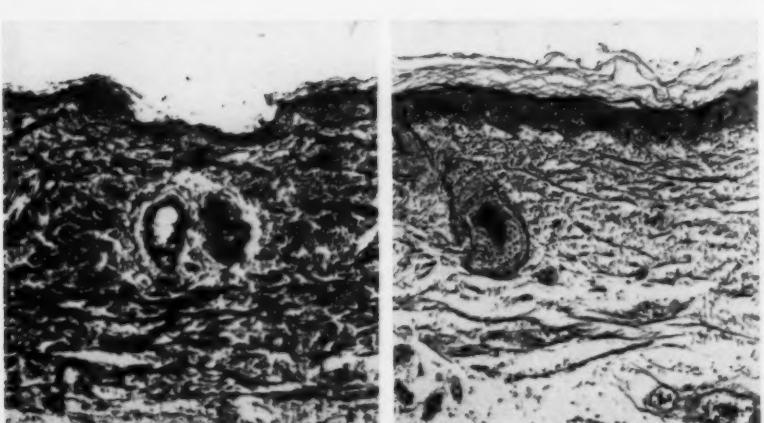


FIG. 6. Control, Gomori (3) silver stain of skin, from infant weighing 1500 grams. Virtually all the dark staining structures of the corium are not argyrophilic but have the usual reddish color of adult collagen.

FIG. 7. Section, Gomori (3) silver stain, from Case 2, same magnification as Figure 6. Note delicacy of fibrils in corium. Such fibrils are truly argyrophilic and hence conform with the definition of reticulum.

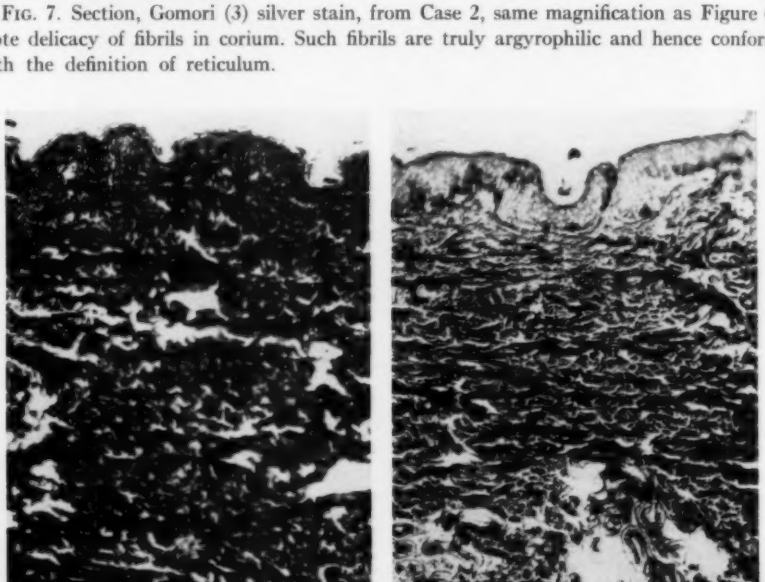


FIG. 8. Control section, Gomori (3) silver stain, from a 2 year old child. Note thick extremely dark staining (reddish actually) collagen bundles.

FIG. 9. Section, Gomori (3) silver stain, from Case 3, same magnification as Figure 8. Note difference in size of fibers. All of the dark staining ones are argyrophobic. Only a few of the fibers beneath the epithelium stain black with the silver preparation.

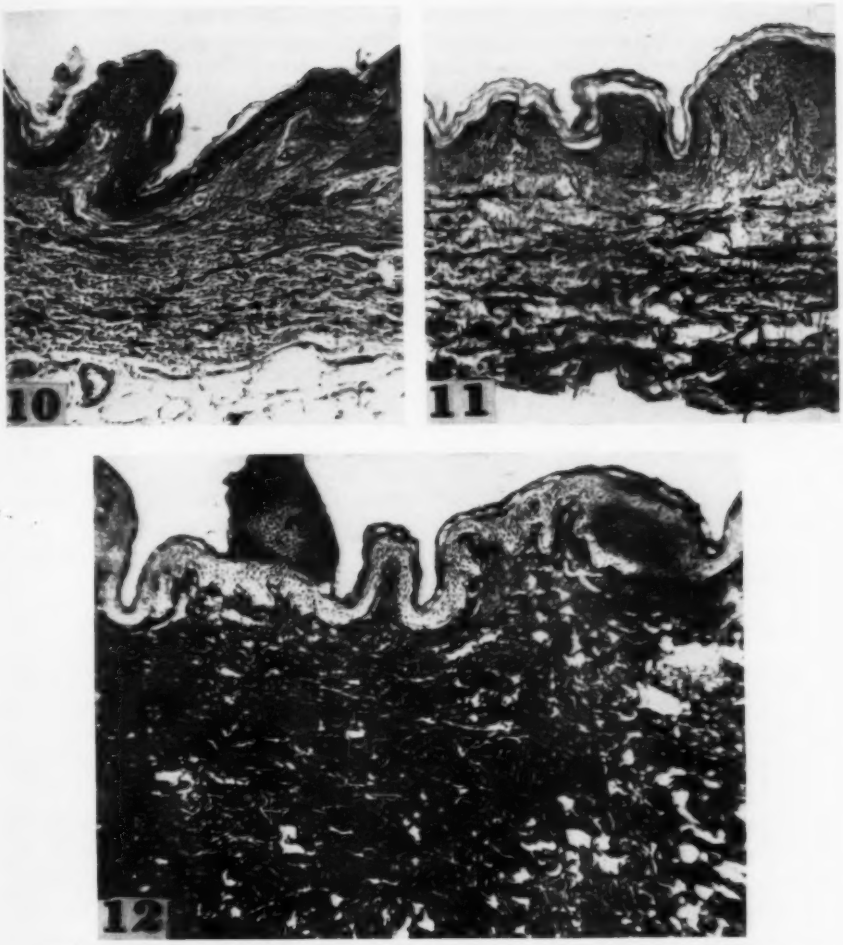


FIG. 10. Section, Gomori (3) silver stain, from Case 4. Note delicacy of fibers in comparison with those of Fig. 12, skin from a normal adult. There are only a few argyrophilic fibers present. Note great difference in thickness.

FIG. 11. Section, Gomori (3) silver stain from Case 5. The fibers are wider than those of Figure 10 but not so prominent as those of the control (Figure 12). There are argyrophilic fibers beneath the epithelium.

FIG. 12. Control, Gomori (3) silver stain, from normal individual. Note great thickness of corium and size of collagen bundles.

stains revealed a delicacy of the fibers not apparent in the hematoxylin and eosin preparations. So too, argyrophilic fibers were present, while only argyrophobic structures were found in the control of this same age.

Case 4 (*S.P. 52–1406*). A 60 year old white, deaf female was admitted with a fracture and

displacement of the left patella following a fall. Positive findings were: atrophic changes in the skin over the arms and legs, deep silver-blue color of sclerae, pigeon breast and paroxysmal tachycardia. No bony abnormalities were seen. Further questioning elicited history that patient's father and her sister had blue sclerae and that a daughter and grand-daughter did also. In addition, her sister's daughter and her daughter exhibited this abnormality.

At operation the lower fragment of the fractured patella was removed. A biopsy of skin and subcutaneous tissue was taken for which we are grateful to Dr. Victor A. McKusick. Sections (Fig. 10) of the latter revealed normal epithelium. There was some decrease in width of the corium. Silver stain revealed a definite increase in number of argyrophilic fibers over the normal (Fig. 12). Collagen bundles were present but were not so numerous or so dense as those noted in the normal control. Periodic acid-Schiff preparations revealed thin positive fibers in contrast to the non-PAS positive controls.

Case 5 (*HSK1*). A 36 year old colored female had had numerous fractures during her life. On examination blue sclerotics and maldevelopment of her teeth were noted. The skin was thought to be thinner than normal. She had been blind in the right eye for 17 years. This structure was removed and at the same time a skin biopsy performed by Dr. Albert D. Ruedemann, Jr. of the Wilmer Institute.

Microscopically, the epithelial layer was normal (Fig. 11). The corium was less wide than normal. The fibers, on the whole, appeared thinner than those of the control. Silver stain showed that some of the thin fibers, particularly those just beneath the epithelium were argyrophilic. The thicker structures beneath were argyrophobic.

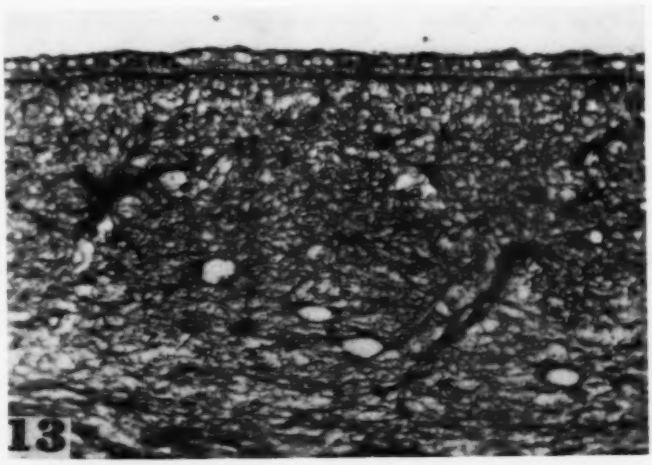
Strips of this skin were heated gradually in the way the skin of Case 1 was studied. Contraction of the collagen occurred at 67°C.

DISCUSSION

From the character of the abnormalities in the bones, skin, and ocular tissues which had been described previously (1), the designation of osteogenesis imperfecta as a "connective tissue diathesis" seemed appropriate. The changes in corium in the 4 additional cases herein reported would tend further to substantiate this concept.

As was pointed out elsewhere (1), the idea that the osteogenesis imperfecta syndrome results from a fundamental disturbance in connective tissues in certain areas is not a new one. K. H. Bauer (6) in 1920 pointed out that osteogenesis imperfecta represented a "disfunction of all ground substance producing cell elements." This concept was also discussed by Jeckln (7) who used the term "mesynchymal exhaustion" (Erschopfung). Neither Bauer or Jeckln noted any particular changes in the corium. Their attention was directed primarily on the skeletal tissues and teeth. However, abnormality of the skin in cases of osteogenesis imperfecta has been noted. Blegvad and Haxthausen (8) described the microscopic appearance of the corium in a case of "blue sclerotics and brittle bones." The corium showed atrophy and cellular infiltration along the vessels. Seedorf (9) called attention to thin skin clinically in cases of osteogenesis imperfecta. Gruber (10) has described the microscopic appearance of embryonic corium in a case of osteogenesis imperfecta congenita. Grossly the skin appeared thin and microscopically, the corium did not have a parallel-fibered

structure. Instead, there was a thin filamentous, reticular, spongy tissue. This is certainly similar to what we shall call attention to in Figure 13.



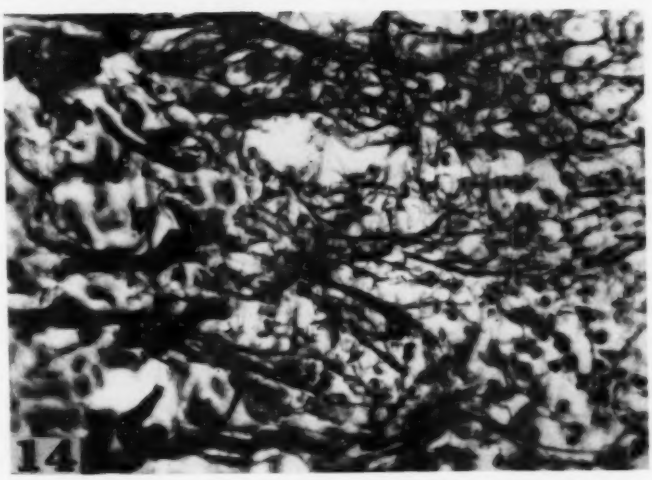


FIG. 13. Section of skin, Gomori (3) silver stain, from 3 month fetus. The tissue beneath the epithelium is composed of a loose, spongy, network of argyrophilic fibrils.

FIG. 14. Section, Gomori (3) silver stain, through a healing skin incision. Field is from area where new connective tissue fibrils are appearing. Fine, newly formed, argyrophilic fibrils are merging with broader, non-argyrophilic more mature appearing collagen fibers.

A recent report by Scott and Stiris (11) dealing with scar tissue formation in individuals exhibiting osteogenesis imperfecta is of great interest. A statisti-

cal tendency for the development of wide or hypertrophic scars was present in those having manifestations of skeletal disease in contrast to a control group. It would be most interesting to study such healing wounds microscopically in order to follow the pattern of connective tissue proliferation, particularly with respect to reticulum maturation.

It is not necessary to go into the old and involved reticulum *versus* collagen controversy because today, as a result of studies with the electron microscope it would appear that these two fibrous proteins are identical (12, 13). This concept was, of course, foreshadowed by Wolbach's (14) studies of the organization of blood clots in healing scorbutus. Similar changes are seen whenever new connective tissue fibers appear, as, for instance, in a healing skin wound (Fig. 14). The first fibers to make their appearance are thin argyrophilic ones. Such structures stain intensely with the PAS technique. The property of silver staining has been used for many years to differentiate between reticulum and collagen. The physicochemical differences responsible for argyrophilia are not as yet understood (12) nor is it clear why reticulum gives a positive PAS reaction.

Studies of the embryonic development of the corium make the defect in osteogenesis imperfecta a little clearer. If the skin from a 3 month's old fetus is examined microscopically (Fig. 13), a loose reticular structure is found beneath the epithelial layer. This contains blood vessels and lymphatics. Silver stain shows that the fibers are argyrophilic; not a vestige of argyrophobic material is present. Such fibers stain intensely with the PAS technique. Gross (12) has demonstrated that such reticulum fibers have the characteristic 640 Å banding of collagen. As the organism matures these argyrophilic fibers grow larger and lose their capacity to stain with silver. Hence by definition they become collagen fibers. It is this lack of maturation or differentiation, whatever the basic physicochemical changes may be, which appears to characterize in varying degrees the osteogenesis imperfecta syndrome as far as the corium is concerned.

It has been realized for many years that one encounters this syndrome in all degrees of severity. Hence since the bones show varying extent of involvement it is not surprising for the skin to be affected differently, too.

The shrinkage temperature, i.e., the temperature at which collagen contracts, has been used for many years by the leather industry as an index of tanning (15). Collagen which has been modified by formaldehyde or chrome salts contracts at a higher temperature while other types of treatment may lead to contraction at lower temperatures. The contraction of the skin of Case 1, that is the contraction of the reticulum fibers at 66°C. only serves to confirm the physical and chemical similarities of these structures to adult collagen.

Changes such as this are not seen in other skin diseases. One immediately thinks of the Ehlers-Danlos syndrome (16); this differs, however, by the presence of excess elastic tissue together with a decrease in *normal-sized* collagen

fibers. So too, the corium which has been examined from exposed areas in patients with senile elastosis, shows increased elastic fibers in addition to looser thinner connective tissue fibers. Dermis from unexposed sites in senile patients is said (16) to be normal.

SUMMARY

A microscopic study of the skin from 5 cases, one previously reported, which exhibited the osteogenesis syndrome has been presented. In all, particularly those in which the skeletal tissues were most affected, the corium showed a characteristic abnormality: failure of argyrophilic reticulum fibrils to become transformed into adult argyrophobic collagen fibers.

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TRANSVERSE LINES IN BONE: THE MECHANISM OF THEIR DEVELOPMENT*

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Early in the application of the x-ray to medicine, transverse lines were noted in growing bones. Excellent illustrations of them are found in the book by H. A. Harris, and their natural history is there completely described (1). Their etiological relationship to acute illness has been noted by many observers and is made certain by some of Harris' case studies. Eliot, Souther and Park (2) studied their structure and pointed out the principle underlying their formation. These workers showed that these *lines* actually are thin strata in the cancellous tissue that result from a cross-branching of the trabeculae in a horizontal plane. We shall refer to them as *lines* because of accepted usage, but shall treat them in our discussion as if they had three dimensions, which of course they have. They have always been regarded as being caused by arrested growth, comparable to the rings in trees or the scales of fish. We undertook to study their development experimentally in the rat because the mechanisms underlying their formation have never been adequately explored.

For these studies we used the so-called single-food-choice technique for studying the nutritive value of various food-stuffs (3, 4). A word of explanation may be given here as to how we came to use this technique for the study of transverse lines. In a study of the nutritive value of alcohol, the diet of 120 day old rats was restricted to a solution of alcohol (30%) and dextrose (30%). The rats lost weight at a steady rate and died after 30–40 days with no signs of deficiency except for their marked loss of weight. At autopsy it was found that all fat had disappeared, and that the muscles had become highly atrophied and in many instances highly transparent. The glands of internal secretion and the nerves seemed little affected (5). Of particular interest for the present purposes was the finding that longitudinal bone growth had practically ceased. The proliferative cartilage was reduced to a thin band; its growth was either stopped or reduced to a barely perceptible amount; and the trabeculae of the cancellous tissue immediately under the cartilage were entirely resorbed, only those at the sides remaining. Thus, longitudinal bone growth had virtually ceased at a stage in an animal's life when normally bone growth proceeds at the most rapid rate.

It occurred to us then that should these animals show rapid growth on return to normal diet, the condition of complete cessation of bone growth could serve

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as a basis for the study of bone growth, especially with respect to the formation of the transverse lines. Our tests showed indeed that these starved rats actually do grow at a very rapid rate after returning to the stock diet, quickly regaining their normal weight; and that bones taken from animals at intervals after the start of the recovery diet clearly reflect the changes produced by growth.

METHOD

The single-food-choice technique has been described in detail in previous papers (4). It will suffice to state here that the rats were kept separately in cages 11 x 5 x 3.5 inches that contained a non-spillable food-cup and one or two graduated inverted water bottles for fluids. The floor of the cage was made of wire cloth with a ½ inch mesh which allowed feces to drop through freely to a pan several inches below, beyond reach of the rats, thus preventing coprophagy.

Records were made daily of the intake of the solids and fluids; the rats were weighed weekly and whenever a change was made in the diet.

In these experiments powdered dextrose and a 0.02% solution of thiamine chloride were offered in separate containers in place of the alcohol-dextrose solution which was offered in a single container. This change was made because rats of the standard weight (120–149 gm.) live longer (a total of 70–80 days) on vitamin B_1 solution and dextrose than on alcohol-dextrose solution (a total of 35–45 days) (5); and because it seemed possible that the recession of bone growth might be even more severe after an additional 30–40 days.

A total of 45 rats were used in these experiments. Their ages ranged from 23 to 180 days at the start of the deficient diet. We are reporting in detail on only 7 rats since their records were obtained under strictly comparable conditions and are typical for the rest.

These 7 rats were started in cages on our stock diet[1] at an average age of 47 days and an average weight of 92 gm. They remained on the stock diet until they reached weights between 130 and 156 gm. then on the dextrose-vitamin B_1 diet for 56–58 days. From previous experiments we knew that by this time bone growth had definitely decreased. Some animals were killed at once; others were placed on stock diet and killed after various intervals ranging from 2 to 25 days. At autopsy the tibia, femur, and ribs were removed, cleaned and placed in formalin, then sectioned and stained with H & E. Most of our studies were made on the upper tibia, but observations were also made on the other bones.

In other experiments rats were given an exclusively fat diet instead of dextrose and vitamin B_1; the effects of hypophysectomy on bones of 6 rats on our stock diet were also studied.

RESULTS

Effects of the Dextrose-Vitamin B_1 and Recovery Diets on Body Weight, Food and Water Intake

Fig. 1 presents a typical record of a rat on these diets, illustrating the changes in body weight, food and water intake. The ordinates represent body weight and food intake in grams and fluid intake in cc.; the abscissas age in days. This figure shows the records for 20 days on the stock diet; 60 days on the dextrose-

[1] Graham flour 72.5%, casein 10.0%, skim milk powder 10.0%, butter 5.0%, calcium carbonate 1.5% and sodium chloride 1.0%.

vitamin B_1 diet; and 30 days on the recovery diet. At the start of the dextrose-vitamin B_1 diet this rat weighed 147 gm.; on this diet it lost weight at a steady rate until at the end of 60 days it weighed only 88 gm., representing a 40% loss; on the recovery diet it gained at a very rapid rate attaining its starting weight level in 8 days and almost doubling its weight in 15 days.

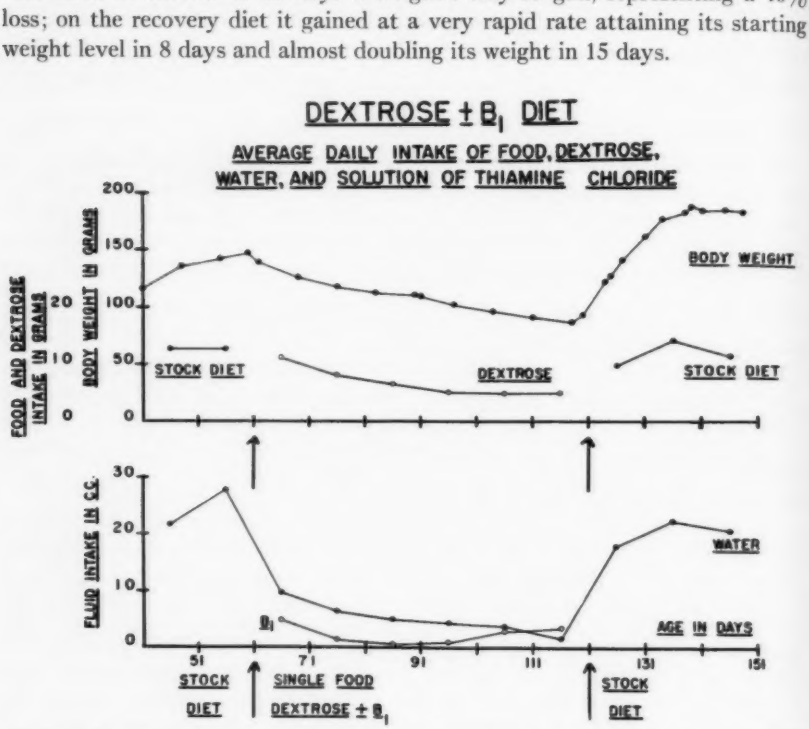

FIG. 1. Typical record of single-food-choice experiment. The ordinates show average daily intake of food, powdered dextrose, thiamine chloride (0.02% solution), and water; and average body weight. The abscissas show time in days for 20 days on the stock diet, 60 days on the dextrose-vitamin B_1 diet, and 30 days on the stock diet (recovery diet).

For the initial 20 days on the stock diet, the rat ate 13 gm. per day on the average. In the next 10-day period, on the single-food-choice diet, it ate almost as much dextrose as it had previously eaten of the stock diet. After that, dextrose intake gradually decreased, reaching a flat low level of 4 gm. in 30 days. During the first 10 days on the recovery diet the rat ate about 10 gm. per day, a little less than before. In the second 10-day period, its food intake increased above the previous average; in the third, when it apparently had regained its full weight, its intake decreased somewhat.

The lower chart shows that for the last 10 days on the stock diet the rat's

daily water intake averaged 28 cc. On the single-food-choice diet its water intake dropped sharply to a much lower level, finally reaching a daily average of about 1 cc. during the period 50–60 days after the diet change. During this interval the rat daily drank from 1 to 4 cc. of the thiamine chloride solution. On return to the stock diet it quickly increased its water intake to a normal level.

Description of the Bones After 50–60 Days on the Dextrose-Vitamin B_1 Diet

The changes that occurred in the bones are illustrated by the photographs in Fig. 2, taken from sections of the upper tibia. Fig. 2A shows the wide band of proliferative cartilage and the large amounts of cancellous trabeculae in a bone from a rat on a normal diet. Fig. 2B shows a bone from a rat that was 55 days old at the start of the dextrose-vitamin B_1 diet and 110 days old when it was sacrificed. The rat had thus been on the experimental diet for 55 days. The cancellous tissue of the shaft has been resorbed until most trabeculae have disappeared and those that remain are minute. The proliferative cartilage has become narrow, 0.144 mm. thick as compared to 0.272 mm. for the tibia from the rat on the normal diet. Although the cartilage was so thin as to indicate complete cessation of growth, still it must have continued to grow at a greatly reduced rate since capillaries and osteoblasts can be seen invading an occasional cartilage cell on the under surface of the plate. By the time the stage of extreme slowing of the cartilage growth had occurred, a thin covering of bone had developed along the entire undersurface of the cartilage plate, invariably thicker at the sides than in the center. Indeed, at the sides it was a solid encasement except for occasional perforations for blood vessels which probably prevented bone formation around them. See Fig. 2C which gives a high power view of part of the section seen in Fig. 2B. Centrally, the bone was fragmentary, showing patches with gaps, the latter occupied by capillaries. It is this bony encasement of the undersurface of the cartilage plate, thicker and nearly continuous peripherally but thin and full of apertures centrally, that represents the transverse line, formed as cartilagenous growth slows to a virtual halt. It was on this the osteoblasts built, when the recovery diet was substituted, as described presently.

In the rib, during the dextrose-vitamin B_1 diet period, the bony encasement of the undersurface of the cartilage plate was comparatively thick and showed only occasional stomata.

In the upper humerus, lower femur, lower radius and ulna, the same conditions prevailed as described in the upper tibia. In the rat there is no epiphyseal cartilage in the lower tibia and lower humerus and the one at the head of the femur is not suitable for study.

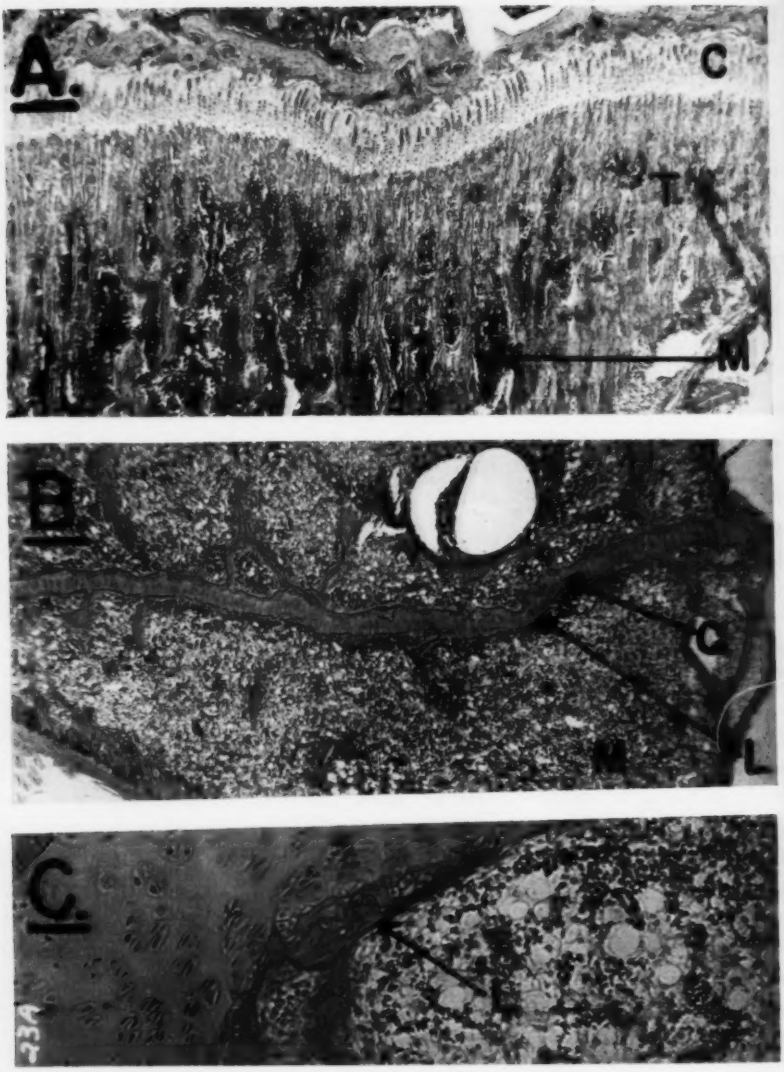

FIG. 2A. Photograph of section of upper tibia of a 76 day old rat on the stock diet.

C—Cartilage
T—Trabeculae
M—Marrow

FIG. 2B. Photograph of section of upper tibia of rat on dextrose-vitamin B_1 diet 56 days; loss in weight 71 gm. or 49%; 110 days old at death.

C—Cartilage
L—Encasement
M—Marrow

The cartilage has been reduced to a narrow band. One can see the discontinuous encasement of bone along the cartilage plate which was formed during the period on dextrose and vitamin B_1 diet.

FIG. 2C. Photograph showing higher magnification of cartilage encasement by bone.

L—Encasement

Description of the Bone and of the Transverse Line at Various Intervals After the Start of the Recovery Diet

Observations on the evolution of the transverse lines were made on many rats of different ages, but it will suffice here to describe one group of rats, since the development was alike in all. The group of 7 rats on the dextrose-vitamin B_1 diet chosen for illustration were 53 to 72 days in age and 130–156 gm. in weight when placed on the dextrose-vitamin B_1 diet. They were kept on the dextrose-vitamin B_1 diet 56 to 58 days, and had lost from 62 to 72 gm. at the end of that time. They were killed 2, 7, 9, 13, 16, 20 and 25 days after the restoration of the full diet.

Fig. 3A and B show photographs of the section of the upper tibia of a rat, killed after 2 days on the recovery diet. The proliferative cartilage measured 0.127 mm. in thickness. The bony film formed on the undersurface during the dextrose-vitamin B_1 period was already covered with a single layer of osteoblasts and a very thin layer of newly formed bone could be identified on its undersurface, since it had the staining properties of osteoid. Thus at the end of 2 days on the recovery diet, new bone formation had commenced, and the rat had gained 12 gm. Fig. 3C shows the section of the upper tibia of a rat killed after 7 days on the recovery diet. The band of proliferative cartilage now measured 0.171 mm., so that it had evidently begun to grow. It is probable that mitoses in the cartilage would have been seen if this, and other sections, had been thinner and better stained for this purpose. The band of bone, i.e. the *line*, was now 0.018 mm. in width. The film of new bone on its undersurface was covered with a blanket of large osteoblasts which appeared in the section as a row of closely packed large cells. Capillaries accompanied by osteoblasts could be seen in the apertures in the *line* but had not penetrated the cartilage beyond. During the 7-day recovery period this animal regained 30 of the 62 gm. it had lost.

Fig. 4A shows the section of the upper tibia of a rat killed after 9 days on the recovery diet. The cartilage had grown further and now measured 0.176 mm. and the *line* bordering its undersurface measured 0.018 mm. Its undersurface was covered by a row of large osteoblasts and osteoid formation was clearly demonstrated. Capillaries and attendant osteoblasts again could be identified in the apertures of the *line*, but had not yet penetrated into the substance of the cartilage. During the 9 days this animal had gained 51 gm., its initial loss being 69 gm.

Fig. 4B shows a section of upper tibia of a rat killed after 13 days on the recovery diet. The cartilage appears in full proliferation, measuring 0.308 mm. The *line* now measures 0.028 mm. (0.084 mm. laterally) in thickness. Capillaries and osteoblasts have penetrated into the cartilage a considerable distance ahead of the *line*, and are forming trabeculae of bone in the normal vertical

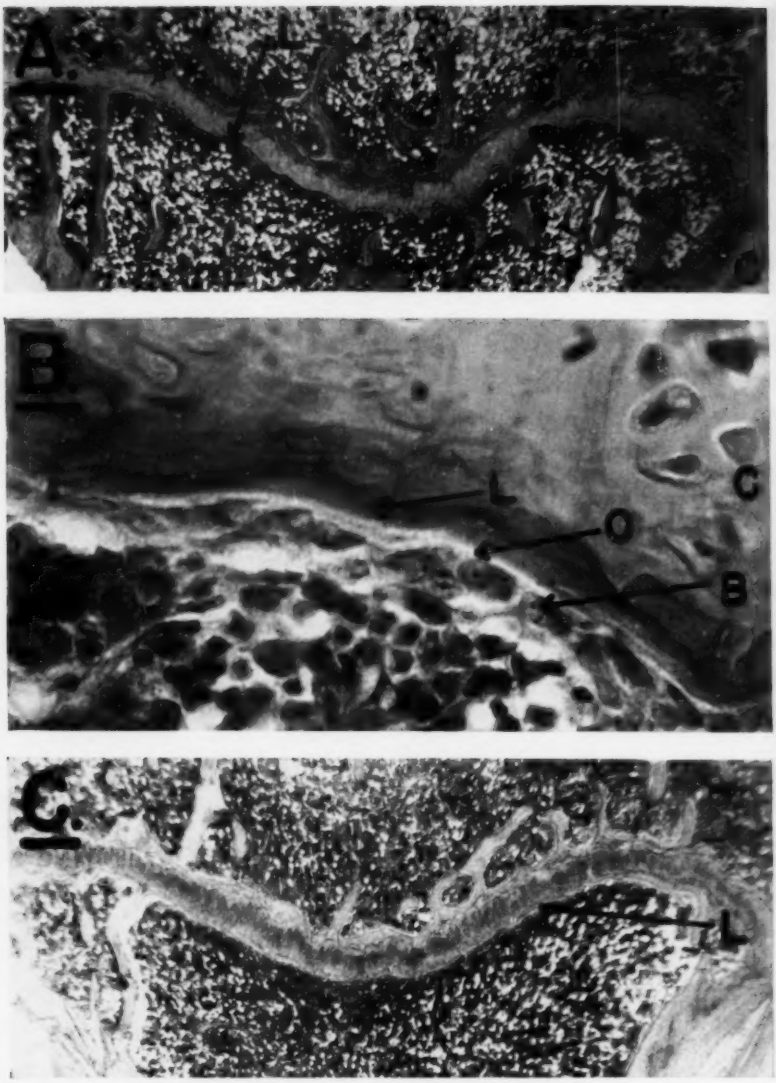

FIG. 3A. Photograph of upper tibia of rat on dextrose-vitamin B_1 diet 58 days; weight loss 72 gm. or 46%; on recovery diet for 2 days; weight gain 12 gm. or 14%; 125 days old at death.

C—Cartilage
L—Transverse line

Osteoblasts have already begun new bone formation in pre-existing bony tissue formed during the dextrose-vitamin B_1 diet. The transverse line has begun to thicken.

FIG. 3B. Same but with higher magnification.

C—Cartilage
L—Transverse line
O—Osteoid
B—Osteoblast

Within the 2 days osteoblasts have appeared on undersurface of *line* to deposit osteoid.

FIG. 3C. Section of upper tibia of rat on dextrose-vitamin B_1 diet 58 days; weight loss 62 gm. or 47%; on recovery diet 7 days; weight gain 30 gm. or 43%; 137 days old at death.

L—Line

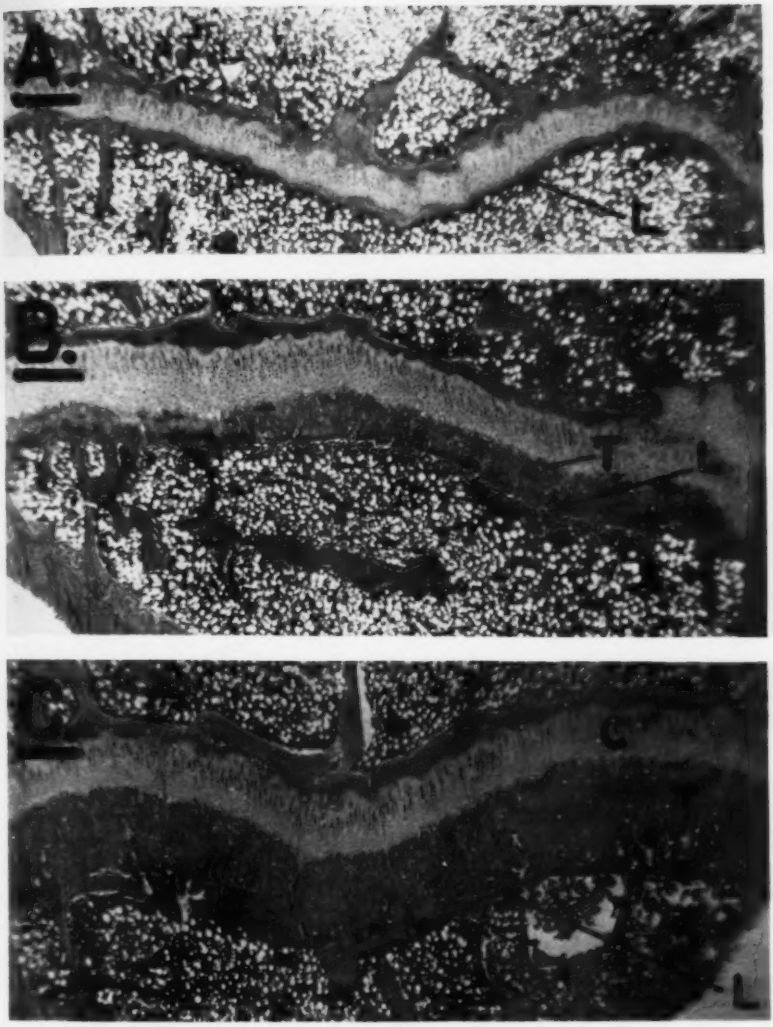

FIG. 4A. Photograph of section of upper tibia of rat on dextrose-vitamin B_1 diet 58 days; weight loss 69 gm. or 49%; on recovery diet 9 days; weight gain 51 gm. or 72%; 136 days old at death.

L—Line

FIG. 4B. Photograph of upper tibia of rat on dextrose-vitamin B_1 diet 58 days; weight loss 70 gm. or 45%; on recovery diet 13 days; weight gain 67 gm. or 80%; 140 days old at death.

T—Trabeculae
L—Transverse line

The cartilage has grown away from the *line*.

FIG. 4C. Photograph of upper tibia of rat on dextrose-vitamin B_1 diet 58 days; weight loss 63 gm. or 48%; on recovery diet 16 days; weight gain 77 gm. or 115%; 140 days old at death.

C—Cartilage
T—Trabeculae
L—Transverse line

The cartilage is still farther away from the *line*.

direction. The *line* now lies 0.252 mm. below the lower border of the proliferative cartilage, i.e. the proliferative cartilage has grown away from it that distance. Osteoblastic growth on the undersurface of the *line* is beg'nning to cease. The loss in weight of this animal on the dextrose-vitamin B_1 diet was 70 gm.; and the gain of weight on the recovery diet 67 gm. during the 13 days.

Fig. 4C shows the section of the upper tibia of a rat killed 16 days after the recovery diet was commenced. The proliferative cartilage measures 0.292 mm. in thickness and the *line* is 0.032 mm. wide. The proliferative cartilage is now separated from the *line* by 0.453 mm. Between it and the cartilage are the vertically disposed trabeculae formed by the osteoblasts on the calcified matrix frame which the cartilage has left behind. The *line* is intact at the sides but has been resorbed centrally. Osteoblastic activity on the undersurface of the *line* is in evidence, but in some locations the osteoblasts have disappeared entirely or have assumed a flattened form. Obviously, new bone formation on the undersurface of the *line* has practically ceased. The animal gained during this 16 day period 77 gm., 14 gm. in excess of the weight lost during the dextrose-vitamin B_1 diet administration.

Fig. 5A shows section of upper tibia of rat killed 20 days after inception of the recovery diet. The proliferative cartilage of 0.279 mm. is not quite as wide as in the two previous animals because growth had slackened. The *line* is almost continuous at the sides but in the center it has been broken up into bits, each bit capping a trabecula. The breaks in continuity are due to the blood vessels and their attendant osteoblasts, passing upwards into the cartilage, and these are much more numerous centrally than at the sides. The beginning of focal resorption of the vertically disposed trabeculae between the *line* and the undersurface of the proliferative cartilage can be seen, and osteoblastic activity on the undersurface of the *line* has ceased. In various places the marrow seems to lie in direct contact with it. The initial loss in this animal during the dextrose-vitamin B_1 diet was 72 gm.; the gain during the 20 days on the restored normal diet was 75 gm.

Fig. 5B shows a section of the tibia of a rat killed 25 days after restoration of the normal diet. The cartilage measures 0.280 mm. The *line* has been almost completely resorbed except for fragments which lie far down the shaft of the bone.

In the other long bones (the lower femur, the upper humerus, and the lower radius and ulna) the line formation during the recovery diet corresponded almost exactly to the above description of the tibia. Longitudinal growth rates in the ends of these bones seemed to correspond to the growth rate in the upper tibia.

On the other hand the rib behaved differently. On the single food diets the development of the *line* in the rib was greater, i.e., it attained considerably

FIG. 5A. Photograph of upper tibia of rat on dextrose-vitamin B_1 diet 58 days; weight loss 72 grams or 55%; on recovery diet 20 days; weight gain 75 gm. or 127%; 140 days old at death.

C—Cartilage
T—Trabeculae
L—Transverse line

FIG. 5B. Photograph of upper tibia of rat on dextrose-vitamin B_1 diet 56 days; weight loss 70 gm. or 51%; on recovery diet 25 days; weight gain 94 gm. or 138%; 134 days old at death.

L—Transverse line

The transverse line though fragmentary, can be clearly seen quite far down the shaft.

FIG. 6A. Photograph of upper tibia of rat on olive oil diet 38 days; weight loss 117 gm. or 57%; on recovery diet 10 days; weight gain 58 gm. or 65%; 101 days old at death.

C—Cartilage
T—Trabeculae
L—Line

The transverse line is beautifully shown. The proliferative cartilage is in full growth. The blood vessels and accompanying osteoblasts have penetrated some distance in advance of the *line*, forming irregular trabeculae from the matrix framework which the cartilage has left behind in the course of the growth.

FIG. 6B. Photograph of upper tibia of rat hypophysectomized at 44 days; killed 41 days after hypophysectomy; weight gain 12 gm. or 19%.

L—Transverse line

The transverse line in the process of formation through cross-branching is well shown. The proliferative cartilage is still growing, though at a very slow rate as shown by the presence of the large cells just above the line. The line has all the features seen in lines in the bones of children. It is obviously formed as longitudinal growth receded.

greater thickness than in the long bones. It was also resorbed much more rapidly, disappearing regularly as early as the 13th day after the recovery diet had been commenced.

Similar results were obtained in a few experiments with an exclusively fat diet which produced cessation of growth more rapidly than the dextrose-vitamin B_1 diet, while forming the transverse line in exactly the same way. See Fig. 6A.

We also have produced transverse lines by means of hypophysectomy. See Fig. 6B.

DISCUSSION

The formation of the transverse line occurs for two reasons: first, the growth of osteoblasts and of cartilage cells are separate and under different regulatory mechanisms; and second, the osteoblast is, functionally speaking, a dependent cell. Figuratively speaking, the osteoblast has no architectural ability; it is a common laborer. All it can do is build bone by a layering process on some preexisting structure, either cartilage matrix or previously formed bone. It can build bone in any direction, as determined by the conformation of material on which it builds.

The architecture of the bone is determined by the cartilage. In the framework of matrix substance of the proliferative zone of cartilage one can discern the future design of the trabecular network of the shaft destined to form from it. The reason that the trabeculae of bone in the shaft are normally disposed vertically is that the structure of the proliferative cartilage is vertical. The walls of matrix substance really form hollow cylinders, set vertically, on the walls of which, inside and outside, the osteoblasts settle and form bone, after having destroyed the contained cartilage cells.

For transverse line formation to occur all that is required is that the growth of the cartilage stops or slows, while the osteoblasts of the shaft continue their work of bone formation. With the stoppage of growth the under surface of the cartilaginous plate becomes an impenetrable ceiling, so to speak, to the osteoblasts and capillaries, and the osteoblasts settle on this ceiling so that the bone formed by them on its undersurface is perforce in the horizontal direction.[2] Our experiments indicate that the transverse line forms while the growth of the cartilage is approaching a standstill; and that the osteoblasts continue to form bone after cartilaginous growth has become so reduced that further invasion no longer occurs significantly. The transverse line basically is thus formed during the period of recession in longitudinal growth.

When the full diet is restored or hypophysectomized animals are treated with the growth hormone, the *line* thickens. The thickening seems to be the result of a lag between the timing of the cartilage cell and of the osteoblast

[2] It is assumed that the end of the bone under description is standing upright.

growth mechanisms. Under the fresh nutrient conditions resulting from the restoration of the full diet, the osteoblast begins to proliferate within 48 hours, and to manufacture matrix. At the same time the cartilage cells also proliferate. But, whereas the osteoblasts can immediately begin to layer matrix on the undersurface of the *line* already formed, the cartilage cells are obliged to go through a preliminary cycle of development. The mother cartilage cells must divide, the offspring cells further divide, then undergo a maturation process in the course of which they become arranged in columns, swell, and finally become senescent. This senescent state must be attained before the osteoblasts and capillaries of the shaft can break into the cartilage and again form bone, as under normal conditions. Thus there is a delay before the osteoblasts and capillaries can break through the *line* into the cartilage beyond, and during this delay the osteoblasts keep thickening the *line*. When the cartilage matures to the point that the capillaries and osteoblasts can break into it, they pass through the apertures of the *line* into the cartilage, creating new bone; and as they do so, the front of active growth advances through the *line* into the growing cartilage beyond. Soon after the capillaries and osteoblasts have penetrated beyond the *line*, thickening of the *line* begins to cease. When the *line* is destroyed it is by the ordinary processes of cellular resorption.

We do not know why the *line* is resorbed in some instances and in others is left behind as longitudinal growth is resumed. We do not know why the *line* is thicker in the rib during the period of partial starvation than in the other long bones, or why in that bone it resorbs earlier when the recovery diet is given.

The *line* discussed to this point, which we have produced experimentally, is composed entirely of bone. The transverse lines commonly encountered in children are not composed entirely of bone but contain rests of calcified matrix substance of the cartilage. In our rats the *line* was composed entirely of bone because partial starvation was carried so far; i.e. the dietetic conditions were so drastic, that virtually all the trabeculae that normally protrude like stalactites from the cartilaginous plate were removed. These incipient trabeculae are composed of calcified cartilaginous matrix. The rests of calcified matrix found in the transverse lines occurring in children prove that the *line* must have formed before the cartilage plate was cleared of these cartilaginous trabeculae. In other words, the trabeculae of calcified matrix substance were still present on the under surface of the cartilage plate when the *line* was formed, and their bases containing cartilage matrix, were thus included in the *line*. In some of our more recent experiments with the rat we have indications that transverse line formation, exactly according to the principles described, can occur before growth of the cartilage has reached extreme slowing, suggesting that the lines in children are the result of temporary slowing of growth rather than complete arrest. This again is the subject for further study.

It is apparent from our experiments that there is no specific cause of transverse line formation since it can be produced by any conditions causing a sufficient slowing of growth of the proliferative cartilage. Wolbach noted the occurrence of transverse lines, corresponding exactly to those produced experimentally by us, in his studies of vitamin A deficiency in the rat (6). Foreshadowing our conclusions, Wolbach wrote[3]: "... in vitamin A deficiency there is a very prompt cessation of growth of epiphyseal cartilages which takes place before the animal as a whole has ceased to gain in weight. The transverse stratum of bone that forms is not peculiar to vitamin A deficiency in that it is an almost exact replica of what occurs at the cessation of normal growth, and for a long time I have believed that there was nothing distinctive about the epiphyseal cartilage response. On resumption of vitamin A, the epiphyseal cartilage cells resume their activities and as a result of growth the stratum of bone is left behind. In this sense it is a product of recovery, although the bone is formed as a result of the cessation of cartilage cell activities."

SUMMARY

A study was made of the natural history of the transverse *line* from the time of its formation to its final dissolution.

The formation of the transverse *line* in the long bones of young rats was studied using as a basis the cessation of bone growth produced by feeding a diet of only dextrose and thiamine chloride solution. On this diet the rats survive 70–80 days and lose 50–60% of their original weight.

The experimental diet produces a picture in the bones which includes resorbed trabeculae and a greatly narrowed proliferative cartilage plate, encased in a thin layer of bone, thicker laterally. This thin layer is the transverse *line*.

After the basal state was reached (58 days on diet), in which measurable growth of the proliferative cartilage had ceased, the rats on experimental diets were returned to stock food. They ate this food in large amounts and grew at a rapid rate, more than doubling their weight in 15 days.

After change to recovery procedures, osteoblasts settle on the bony film encasing the cartilage, and produce osteoid so that the *line* widens; the cartilage plate proliferates and capillaries and osteoblasts grow in through the *line* into the cartilage, forming normal vertically disposed trabeculae between the cartilage and the *line*. The *line* later is broken up by blood vessels and is resorbed. The entire process from the time of the formation of the *line* to its final dissolution requires slightly more than 25 days.

The *line* forms because the osteoblasts keep making bone after the cartilage growth has waned or virtually stopped. After the recovery diet is instituted, the *line* thickens because the osteoblasts make new bone on the pre-existing

[3] This quotation is from a personal communication.

bony incasement without delay, whereas time is required for the cartilage cells to reestablish their cycle, a sequence beginning with division and ending in senescence. This cycle must be passed through before the osteoblasts and capillaries can penetrate the cartilage, destroy its cells, and form bone on the matrix frame. The thickening of the *line* ceases when the capillaries and osteoblasts have penetrated the *line* to form bone beyond. In the rat, the *line* is then resorbed.

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ULTRAFILTRATION STUDIES ON CALCIUM AND PHOSPHORUS IN PATHOLOGICAL HUMAN SERUM*

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Many attempts have been made in the past to define the relationship between calcium and protein of the serum in various pathological states in man. The results have not always been in agreement due in part perhaps to differences in the methods employed. These methods have included the frog heart bioassay technique for the determination of ionized calcium (1) and various ultrafiltration procedures (2, 3, 4). In some of the latter pH was not strictly controlled; in others, simultaneous determinations of total serum calcium, phosphorus and protein in various disease states were carried out, and then by mathematical analyses attempts were made to determine the relationship among these serum components (5, 6, 7).

Lest confusion exist regarding terminology, the *ultrafilterable calcium* of serum represents the concentration of calcium in the fluid which may be forced through a semipermeable membrane by the application of pressure to serum enclosed within the membrane. By the *diffusible calcium* of serum is meant that portion of serum calcium which is not "bound" to protein. When the serum is ultrafiltered, it is the diffusible portion which passes into the ultrafiltrate. Nearly all of the diffusible calcium is in ionic form, according to McLean and Hastings (1), with only a small amount (about 0.25 mg. per 100 cc.) being bound in a citrate complex. However, there is a considerable discrepancy between the normal range of ionized calcium as reported by McLean and Hastings (4.25 to 5.25 mg. per 100 cc. at pH 7.35) (1) and the ultrafilterable calcium determined by using the technique which we have employed (6.7 to 7.7 mg. per 100 cc. (Table I) at pH 7.3–7.7). The explanation for this is not clear, but a similar discrepancy has been noted previously by Morrison, McLean and Jackson (7). While it is probable that only the ionized fraction is involved in neuromuscular excitability, the possibility exists that all the ultrafilterable fraction of serum is utilized in the mechanism of calcification.

Certain ultrafiltration studies pertaining to calcium, inorganic phosphorus and protein in pooled human sera, to which various ions had been added in order to simulate pathological conditions, were described in a previous paper from this laboratory (8). The present communication deals with the ultra-

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filtration of serum obtained from normal subjects and from patients with various disorders manifested by abnormalities in serum calcium, phosphorus and protein concentrations. This has been done in order to determine in these disease states the relationships among these serum components, a technique being used which permits more rigid pH control than has been used in other studies previously mentioned.

TABLE I

Ultrafiltration of Serum from Normal Persons

SUBJECT	DATE	SERUM Ca MG. %	FILTRATE Ca MG. %	% FILT. Ca*	SERUM P MG. %	FILTRATE P MG. %	% FILT. P†	SERUM TOTAL PROTEIN GM. %	FILTRATE PH
W. H.	1/2/52	10.1	6.7	66	3.9	4.3	110	7.1	7.5
T. C.	1/7/52	9.9	6.9	70	3.1	3.5	113	7.2	7.4
J. H.	1/7/52	10.2	7.0	69	3.2	3.5	109	7.3	7.5
T. H.	1/8/52	10.0	6.7	67	3.7	4.4	119	6.6	7.5
J. S.	1/24/52	10.3	6.7	65	4.0	4.4	110	6.9	7.4
C. P.	3/6/52	10.4	7.1	68	2.7	3.4	126	7.1	7.4
E. H.	5/14/52	10.7	7.2	67	4.2	4.7	112	7.5	7.5
P. C.	6/20/52	10.4	7.0	67	3.9	4.3	110	6.8	7.5
J. W.	6/20/52	10.4	7.1	68	3.2	3.6	112	6.7	7.5
H. L.	6/20/52	10.3	7.7	75	3.3	3.7	112	7.0	7.5

* $\frac{\text{Filtrate Ca} \times 100.}{\text{Serum Ca}}$

† $\frac{\text{Filtrate P} \times 100.}{\text{Serum P}}$

METHODS

The chemical procedures used were identical with those described in a previous report (8). The technique of Lavietes (9) was used for ultrafiltration which was allowed to proceed for 24 hours at 200 mm. mercury pressure in each instance. The serum was obtained from venous blood which was drawn under oil and centrifuged after being allowed to clot for half an hour.[1] In many cases the serum sample was immediately set up for ultrafiltration; in other instances the serum was kept frozen at −10°F. for varying periods of time (sometimes up to several weeks). There is evidence that such freezing does not alter the calcium-protein relationship (8). All serum, except when specifically stated to the contrary, was saturated with 5% CO_2 in 95% oxygen just prior to ultrafiltration, and the results have been included only when the pH of the ultrafiltrate ranged between 7.3 and 7.7 on the Beckman pH meter at room temperature (25° to 30°C.).

The error of the method for calcium determination (10), when analyses are done in duplicate and the results averaged, is ±0.1 mg. per 100 ml. The error of the phosphorus method (11) is also ±0.1 mg. per 100 ml. The terms "% ultrafilterable Ca" and "% ultrafilterable P" are used to express the *ratio* between the Ca and P concentrations in the ultrafiltrate and in the original serum. This method of expression is accompanied by possible differences in results which vary inversely with the logarithm of the serum calcium and phosphorus con-

[1] Some of the sera in the "normal" group were not taken in the fasting state.

centrations. For example, the % ultrafilterable Ca may vary ±4% at the lowest serum Ca concentration reported herein (4.5 mg.%), whereas at the highest serum Ca concentration (21.2 mg.%) the possible variation would be only ±1%. This difference is even more striking in the case of ultrafilterable P where the possible variation is ±20% at a serum P level of 1 mg.%, but only ±2% at a serum P concentration of 11 mg.%.

RESULTS

Group I—Normal Persons:

Sera from 10 healthy ambulatory persons were subjected to ultrafiltration as controls. The results may be seen in Table I. It should be noted that the serum Ca concentrations range from 9.9 to 10.7 mg.%, the ultrafilterable Ca from 6.7 to 7.7 mg.%. The ultrafilterable Ca ranges from 65 to 75% of the total serum Ca with an average of 68% for these 10 normal sera.

The serum P[2] concentrations ranged from 2.7 to 4.2 mg. %, while the ultrafilterable P[2] ranged from 3.4 to 4.7 mg.%, the latter thus being 109 to 126% of the total serum P with an average value of 113% for the 10 specimens. Therefore, the inorganic P of these sera is for all practical purposes wholly ultrafilterable, since a value of 111% would be expected for a wholly ultrafilterable anion when the Donnan equilibrium effect and the *water content* of the serum of normal protein concentration are considered (8). The significance of percentages higher than this is not understood.

In this report, all instances where % ultrafilterable P is greater than 100% are considered to be within normal limits because of the rather wide variation introduced by this *method of expressing* the relationship of the filtrate P to the serum P.

The serum total protein concentrations ranged from 6.6 to 7.5 mg.%. Albumin-globulin ratios were not determined.

Group II—Patients with Hyperparathyroidism:

Sera obtained from patients with hyperparathyroidism were subjected to ultrafiltration before and after parathyroid adenomata were surgically removed (Table II). It will be noted that before operation, total serum calcium levels ranged from 11.2 to 20.2 mg.%, and the ultrafilterable calcium from 7.4 to 12.9 mg.%. Total serum protein concentration was within normal limits in all but two instances—patient H. C. in whom it was slightly decreased, and G. S. in whom it was initially elevated. Despite the marked variation in level of hypercalcemia, the percentage of ultrafilterable Ca was within the normal range with two exceptions: 1. H. C. tended to have a slightly elevated percentage of ultrafilterable Ca, undoubtedly due to the low total serum protein concentration. This is substantiated by an examination of the data in the pa-

[2] The terms "serum phosphorus" and "ultrafilterable phosphorus" are used throughout the text and tables in this paper to refer to the inorganic phosphorus of the serum and ultrafiltrate respectively.

TABLE II

Ultrafiltration of Serum from Patients with Hyperparathyroidism, Before and After Parathyroid Adenomas Were Removed

PATIENT	HISTORY NO.	DATE	SERUM Ca MG. %	FILTRATE Ca MG. %	% FILT. Ca	SERUM P MG. %	FILTRATE P MG. %	% FILT. P	SERUM TOTAL PROT. GM. %	SERUM NPN MG. %	SERUM ALK. P'TASE, B.U.*	FILTRATE pH	REMARKS
J. P.	555000	11/14/50	14.8	9.9	67	2.3	—	—	7.5	42	5.3	7.6	Preoperative.
		11/16/50	11.7	7.9	67	1.8	—	—	6.6			7.6	1 day after removal of 1 parathyroid adenoma.
		11/20/50	9.8	6.4	65	3.6			7.6			7.6	5 days postoperative.
B. B.	575980	6/27/51	13.0	8.6	66	2.6	3.0	115	6.5	36	10.2	7.5	Preoperative.
		7/ 5/51	9.7	6.7	69	4.1	4.8	117	6.5	24	8.7	7.5	6 days after removal of 1 parathyroid adenoma.
		8/ 1/51	9.6	7.2	75	3.8			6.8			7.5	1 month postoperative.
L. W.	440159	9/ 4/51	11.2	7.4	66	2.7	3.1	115	7.8	38	6.2	7.5	Preoperative.
		9/14/51	9.6	5.4	56	4.1	5.3	129	7.8			7.4	4 days after removal of 1 parathyroid adenoma.
		9/19/51	10.1	6.4	63	2.6	3.0	115	7.2			7.5	9 days postoperative.
		3/23/52	9.4	6.4	68	2.4	3.2	133	7.3			7.5	6½ months postoperative.
G. S.	563600	10/13/51	12.8	7.8	61	3.0	2.8	93	8.1	64	10.1	7.5	Pre-infarction.
		10/20/51	20.2	12.9	64	5.3	5.1	96	8.3	77		7.5	Probable day of spontaneous infarction of parathyroid adenoma.
		10/23/51	10.7	6.7	63	1.7	2.3	135	7.5	75	11.3	7.6	
		10/29/51	8.2	5.0	61	1.6	1.9	119	8.0	47		7.5	
		11/ 3/51	8.3	5.9	71	1.2	1.5	125	6.6	34		7.5	1 day after removal of infarcted parathyroid adenoma.
		2/ 5/52	9.7	6.2	64	2.7	3.3	122	7.0			7.6	3 months after removal of infarcted parathyroid adenoma.
		11/14/52	10.2	7.8	76	3.4	3.5	103	6.4	32		7.6	1 year after removal of infarcted parathyroid adenoma.
J. H.	149997	10/20/51	16.1	11.4	71	3.0	3.2	107	7.0	28	6.5	7.5	Preoperative.
		10/27/51	15.3	10.5	69	2.6	3.1	119	6.7			7.4	Preoperative.
		11/10/51	14.9	10.6	71	2.1	2.4	114	6.6			7.6	Preoperative.
		11/11/51	12.3	8.9	72	1.7	2.0	118	6.1			7.6	1 day after removal of 1 parathyroid adenoma.
		11/15/51	9.6	6.7	70	2.3	2.7	117	6.4	26	7.6	7.5	5 days postoperative.
		12/19/51	9.4	6.4	68	3.8	3.9	103	6.9	32	6.8	7.6	5½ weeks postoperative.
R. C.	515217	12/17/51	12.2	8.5	70	2.2	2.4	109	6.6	30	3.7	7.5	Preoperative.
		6/ 2/52	11.4	8.3	73	2.5	3.0	120	6.6			7.4	5½ months after removal of 1 parathyroid adenoma.
		6/ 5/52	9.1	6.1	67	3.0	3.5	117	6.8			7.4	3 days after removal of second parathyroid adenoma.
		11/ 6/52	10.4	7.1	68	3.6	4.1	114	7.1			7.5	5 months after removal of second parathyroid adenoma.
P. P.	621251	11/ 5/52	13.0	9.7	75	2.4	2.7	113	6.8	21	4.9	7.5	Preoperative.
		11/ 6/52	13.7	9.4	69	2.3	2.9	126	7.5		5.9	7.6	Preoperative.
		11/11/52	13.1	9.2	70	2.2	2.6	118	7.2			7.6	Preoperative.
		11/14/52	9.3	6.8	73	3.2	3.6	113	6.2			7.5	3 days after removal of 1 parathyroid adenoma.
		11/17/52	9.3	6.9	74	3.3	4.0	121	6.4			7.5	6 days postoperative.
		11/26/52	9.3	6.8	73	3.2	3.3	103	6.3			7.5	15 days postoperative.
		1/12/53	9.9	7.4	75	3.1	3.5	113			5.3	7.6	2 months postoperative.
H. C.	623930	11/13/52	13.1	10.0	76	2.1	2.5	119	5.7	27	7.6	7.5	Preoperative.
		11/19/52	13.5	9.9	73	1.7	2.1	123	5.7			7.5	Preoperative.
		11/24/52	9.3	7.2	77	3.6	4.1	114	5.3			7.6	5 days after removal of 1 parathyroid adenoma.
		11/28/52	9.6	7.4	77	3.5	3.8	109	5.5			7.5	9 days postoperative.
A. D.	624279	11/18/52	14.0	9.9	71	2.3	2.7	117	6.8	23	5.2	7.5	Preoperative.
		11/30/52	13.4	9.3	69	1.8	2.1	117	6.7	27	4.5	7.5	Preoperative.
		12/ 6/52	9.5	6.9	73	2.7	3.1	115	6.4		6.4	7.6	4 days after removal of 1 parathyroid adenoma.
		12/10/52	10.7	7.3	68	3.0	3.4	113	6.7			7.6	8 days postoperative.
C. F.	579168	8/11/52	9.9	6.7	68	4.6	5.2	113	6.7			7.6	Control.
		8/16/52	10.3	7.3	71	4.3	4.9	114	6.3	39		7.5	Parathyroid extract 40 cc. (100 U/cc.) i.m. over previous 72-hour period.

* Bodansky units.

tients with hypoproteinemia (Table VII); 2. G. S., in whom the percentage of ultrafilterable Ca tended to be a little low due probably to two factors, viz., dehydration with resultant elevated serum protein concentration and, at the height of the hypercalcemia when percentage of ultrafilterable P was also reduced due to possible formation of a "colloidal calcium-phosphorus-protein complex (8)."

After the hyperparathyroidism was corrected by surgical removal of the parathyroid adenomata, it will be noted that the ultrafilterable Ca fell gradually in direct proportion to the fall in total serum Ca, so that again the percentage of ultrafilterable Ca remained within the normal range in all instances with a slight but insignificant variation. A fall in total serum protein concentration probably accounted for the rise in percentage ultrafilterable Ca in one instance (G. S.).

The ultrafilterable phosphorus was determined in eight of the nine patients. Before operation the percentage of ultrafilterable P was within the normal range in all but one instance (G. S.) which is discussed above.

After operation it will be noted that for the most part the average percentage of ultrafilterable P remained within the normal range with two exceptions, where it tended to be especially high (L. W. and G. S.). The exact significance of this latter finding is unknown at present.

The final subject (C. F.) is included in this group to show the effect of parathyroid extract administration on the serum and ultrafiltrate Ca. This normal subject received a large dose of parathyroid hormone (40 cc. intramuscularly over a 72-hour period). At the end of this period there was but slight rise in total serum calcium and a comparable rise in ultrafilterable calcium so that the percentage of ultrafilterable calcium did not change significantly.

Group III—Patients with Hypoparathyroidism.

The sera of eight patients with hypoparathyroidism (six post-thyroidectomy and two idiopathic) were subjected to ultrafiltration (Table III). In seven of these patients, serum was obtained before any vitamin D therapy was given. All but two of these seven (A. M. and M. M.) had been receiving oral calcium salts for several weeks prior to these studies. None was adequately controlled symptomatically or chemically. Several days prior to the initial ultrafiltration studies in each patient, the administration of oral calcium salts was discontinued. In these seven patients, serum calcium ranged from 4.5 to 7.2 mg.% while the ultrafilterable calcium ranged from 2.9 to 5.3 mg.% or 61 to 74% of the total serum calcium. Subsequent ultrafiltration determinations were made after vitamin D therapy and added oral calcium salts (in some cases) had caused a serum calcium rise. With treatment in each case the ultrafilterable calcium rose as did the total serum calcium, and the ratio between the two remained quite constant. The serum of F. G., who had been under treatment with vitamin D and oral calcium gluconate for eight years, manifested a per-

TABLE III

Ultrafiltration Data on Serum from Patients with Hypoparathyroidism Before and After Treatment

PATIENT	HISTORY NO.	TYPE OF HYPOPARATHYROIDISM	DATE	SERUM Ca MG. %	FILTRATE Ca MG. %	% FILT. Ca	SERUM P MG. %	FILTRATE P MG. %	% FILT. P	SERUM TOTAL PROT. GM. %	SERUM ALB. GM. %	SERUM GLOB. GM. %	FILTRATE PH	REMARKS
A. M.	256843	P.-T.*	2/12/51	5.3	3.8	72	8.3			7.3			7.5	Latent tetany. No treatment.
			6/13/51	7.9	5.4	68	5.6	6.1	109	7.8			7.5	Vitamin D in increasing dosage from 50,000 to 150,000 U. q.d. Oral $CaCl_2$ 14.4 gm. q.d. (for 4 months).
			10/29/52	7.8	5.5	71	5.7	6.3	111	7.4	4.2	3.2	7.6	Vitamin D gradually increased to 350,000 U. q.d. $CaCl_2$ reduced to 6.6 gm. q.d.
O. H.	519127	P.-T.*	11/29/51	4.7	3.0	64	6.8	7.7	113	6.5			7.5	Latent tetany. No treatment.
			11/30/51	4.5	2.9	64	7.5	8.6	115	6.6	4.4	2.2	7.6	Latent tetany. No treatment.
			11/17/52	8.2	6.0	73	6.5	7.2	111	7.3			7.5	Vitamin D in increasing dosage to 200,000 U. q.d. Oral $CaCl_2$ 9.6 gm. per day for 1 year.
M. B.	199305	P.-T.*	3/12/52	5.9	3.6	61	5.6			7.1	4.8	2.3	7.4	Latent tetany. No treatment.
			11/ 7/52	10.1	7.0	69	3.7	4.2	114	7.4			7.6	Vitamin D 100,000 U. q.d. for 8 months.
E. P.	602354	P.-T.*	4/ 8/52	6.1	4.2	69	6.1	6.7	110	6.7			7.4	Latent tetany. No treatment.
			7/ 3/52	12.6	7.7	61	3.3	3.8	115	7.6			7.7	Vitamin D 200,000 U. q.d. and oral Ca lactate 15–30 gm. q.d. for 2½ months. Vomiting from Vit. D intoxication.
			10/ 3/52	9.9	6.7	68	4.7	5.2	111	7.2			7.6	Vitamin D reduced to 100,000 U., then increased to 150,000 U. q.d.
			10/31/52	9.5	6.9	73	4.6	5.0	109	7.0			7.6	Vitamin D 150,000 U. q.d.
A. C.	229986	Idiopathic	4/29/52	6.1	4.3	70	5.5	6.3	115	5.8			7.5	In active tetany. No treatment for 2 days. Only treatment had been oral Ca chloride.
			9/23/52	10.4	7.3	70	4.1	4.5	110	6.2			7.5	After treatment with vitamin D 200,000 U. q.d. decreased to 100,000 U q.d. for 5 months.
F. G.	242307	Idiopathic	5/16/52	7.7	5.0	65	6.0	7.3	121	6.0	4.1	1.9	7.7	No symptoms or signs of tetany. Treated with vitamin D 200,000 U. and oral Ca gluconate daily for 8 years.
B. W.	606798	P.-T.*	5/22/52	7.2	5.3	74	4.7	5.3	113	6.7			7.6	Latent tetany. Intermittent oral Ca salts for 5½ years.
			5/28/52	7.2	5.2	72	5.0	5.9	118	7.4			7.4	Vitamin D 200,000 U. q.d. for 5 days.
			6/ 5/52	8.8	6.2	70	5.1	6.3	124	6.6			7.4	Vitamin D 200,000 U. q.d. for 13 days.
			8/ 1/52	8.6	6.5	76	5.0	5.6	112	7.1			7.6	Vitamin D 150,000 U. q.d. for 10 weeks.
M. M.	592983	P.-T.*	7/15/52	6.7	4.1	61	5.9	6.8	115	7.3	4.5	2.8	7.5	Latent tetany—untreated for 8 years.
			7/21/52	6.2	3.9	63	5.1	5.6	110	7.0			7.5	After ACTH 100 mg. q.d. i.m. for 4 days.
			11/ 4/52	9.6	6.4	67	4.6	5.1	111	7.2			7.6	Oral $CaCl_2$ 4.8 gm. and vitamin D 150,000 U. q.d. for 3½ months.

* Post-Thyroidectomy.

centage of ultrafilterable calcium within the normal range. In all these samples of sera taken before and after treatment, the percentages of ultrafilterable P were within the normal range.

Group IV—Patients with Vitamin D Intoxication.

The sera of two patients with hypercalcemia due to prolonged vitamin D therapy for arthritis were ultrafiltered (Table IV). It may be noted that the serum calcium of patient M. J. was normal when first obtained. This may have been due in part to the associated renal insufficiency, but the authors believe that a more likely explanation is that her previous cortisone therapy may have produced a fall in serum calcium level. There was no remarkable variation in the percentage of ultrafilterable calcium, regardless of the level of serum calcium in several samples of serum from this patient. In patient H. W. the

TABLE IV

Ultrafiltration of Serum from Patients with Vitamin D Intoxication

PATIENT	HISTORY NO.	DATE	SERUM Ca MG. %	FILTRATE Ca MG. %	% FILT. Ca	SERUM P MG. %	FILTRATE P MG. %	% FILT. P	SERUM TOTAL PROT. GM. %	SERUM ALB. GM. %	SERUM GLOB. GM. %	SERUM NPN MG. %	FILTRATE PH	REMARKS
M. J.	575693	7/18/51	10.3	7.0	68	6.2			7.6	4.9	2.7	128	7.4	Vitamin D 450,000 U. q.d. for arthritis for 6 years. 5 weeks prior to 7/18/51, vitamin D stopped, patient received oral cortisone 100 mg. q.d.
		7/25/51	9.4	6.6	70	5.1	3.8	114	6.8	4.3	2.5	72	7.3	No therapy.
		9/ 7/51	12.5	8.5	68	5.2	6.1	117	7.3	4.5	2.8	50	7.5	No therapy.
H. W.	602225	4/ 8/52	18.7	10.2	55	4.9	4.3	88	7.9	5.3	2.6	48	7.5	Vitamin D 300,000 U. q.d. for arthritis for previous 8 weeks; therapy stopped 4/4/52.
		4/16/52	14.0	10.0	71	2.3	3.1	135	7.1			42	7.4	
		5/26/52	11.4	8.3	73	3.1	4.1	132	7.4				7.5	

first serum sample, which was markedly hypercalcemic as well as hyperphosphatemic, reveals reduced ultrafilterability of both Ca and P. It may be seen that as the total serum Ca level fell when therapy with vitamin D was stopped, the percentages of ultrafilterable Ca and P rose to normal in subsequent samples of this patient's serum.

Group V—Patients with Multiple Myeloma.

The sera of eight patients with multiple myeloma was ultrafiltered and the data may be seen in Table V. Hypercalcemia (serum Ca greater than 11 mg.%) was present in seven of the eight patients. Hyperproteinemia (serum total protein greater than 8 gm.%) was found in two of these hypercalcemic patients and was also present in the normocalcemic patient. Two of the hypercalcemic patients had coincident hypoproteinemia (serum total protein less than 6 gm. %). The percentage of ultrafilterable Ca was within the normal range in the sera of five patients, although one of these was hyperproteinemic and another was hypoproteinemic. The percentage of ultrafilterable calcium was high in

• Post-Thyroidectomy.

the sera of two patients (S. C. and E. R.), both of whom had hypercalcemia and hyperproteinemia with marked reversal of the A/G ratio. This latter situation, however, was also present in patient W. E. and a percentage of ultrafilterable Ca within the normal range was found.

Treatment of three of the patients in this group with ACTH resulted in no significant change in the ratio of ultrafilterable to total serum Ca, although the total serum Ca fell to normal in two of the patients while under therapy. In these latter two patients the serum protein did not change significantly in one instance (D. K.) and fell slightly in the other patient (S. C.).

TABLE V

Ultrafiltration of Serum from Patients with Multiple Myeloma

PATIENT	HISTORY NO.	DATE	SERUM Ca MG. %	FILTRATE Ca MG. %	% FILT. Ca	SERUM P MG. %	FILTRATE P MG. %	% FILT. P	SERUM TOTAL PROT. GM. %	SERUM ALB. GM. %	SERUM GLOB. GM. %	SERUM NPN MG. %	FILTRATE PH	URINARY BENCE-JONES PROTEIN	REMARKS
J. W.	U.M.H.	2/17/50	13.4	9.5	71	5.2	5.6	108	6.3				7.4		Stilbestrol 2 mg. q.d. started and continued throughout period of study. Course of ACTH i.m. 3/18–3/25/50. Total dose = 550 mg.
		3/21/50	13.4	9.4	70	5.5	6.1	111	6.7				7.4		
		4/ 4/50	15.5	10.6	68	4.4	5.1	116	6.3				7.5		
V. P.	545723	8/ 8/50	11.9	7.8	66	5.1			5.6	3.8	1.8	87	7.5	+	
		8/10/50	11.9	8.5	71	6.0	6.2	103	5.9	4.0	1.9		7.6		
D. K.	554286	10/31/50	16.2	10.0	62	4.8			5.9	4.6	1.3	86	7.4	+	
		11/13/50	10.5	7.0	67	2.5			6.2	4.6	1.6	77	7.4		After 12 days ACTH i.m. Total dose = 1280 mg.
S. C.	107675	1/ 9/51	12.3	9.5	77	4.0			13.4	3.2	10.2		7.5	−	
		1/20/51	13.7	11.0	80	3.0	3.3	110	12.5	3.0	9.5	32	7.6		
		1/31/51	10.6	7.9	75	3.9			11.1	2.8	8.3	33	7.6		After 10 days ACTH i.m. Total dose = 950 mg.
L. N.	509385	7/21/51	12.8	9.4	73	11.0	12.3	112	6.0	1.9	4.1	82	7.4	−	
W. E.	581259	8/22/51	10.5	7.5	71	3.5	4.1	117	10.8	2.2	8.6	32		+	
E. H.	586037	10/13/51	11.1	7.9	71	7.0	7.7	110	7.4	4.9	2.5	135	7.3	−	
E. R.	622456	11/15/52	14.3	11.2	78	4.5	5.4	120	9.1	3.3	5.8	51	7.5	+	

The serum P was elevated due to renal insufficiency in all but two of the myeloma patients. The percentage of ultrafilterable P in the sera of the seven patients in which it was determined was normal.

Group VI—Patients with Hyperproteinemia Due to Disease States Other Than Multiple Myeloma.

The sera of five patients with hyperglobulinemia due to disease states other than multiple myeloma were ultrafiltered and the results are presented in Table VI. Two of the patients had coincident hypercalcemia, although one was of very mild degree. The serum of only one of the patients (R. L.) showed an increase in percentage of ultrafilterable Ca and it is of interest that this patient had the lowest serum albumin and the highest serum globulin of the group. The serum of another patient (E. B. #517118) had a consistently low percentage of ultrafilterable Ca, perhaps related to the presence of an unusual

protein which may have been also responsible for the greatly increased serum viscosity. In the first serum sample of patient M. W., the finding of a slightly

TABLE VI

Ultrafiltration of Serum from Patients with Hyperproteinemia Due to Disease States Other Than Multiple Myeloma

PATIENT	HISTORY NO.	DIAGNOSIS	DATE	SERUM Ca MG. %	FILTRATE Ca MG. %	% FILT. Ca	SERUM P MG. %	FILTRATE P MG. %	% FILT. P	SERUM TOTAL PROT. GM. %	SERUM ALB. GM. %	SERUM GLOB. GM. %	FILTRATE PH	REMARKS
R. L.	158592	Lymphopathia venereum	3/ 5/52	9.4	7.2	77	3.8			13.5	2.7	10.8	7.5	
L. H.	513861	Pulmonary tbc, with subcutaneous nodules	2/28/52	10.6	7.2	68	5.3	5.9	111	8.7	3.5	5.2	7.6	
E. B.	414566	? disseminated lupus erythematosus	3/17/52	9.5	7.2	76	3.8	4.2	110	9.1	3.9	5.2	7.5	
E. B.	517118	Chronic lymphoid leukemia	2/19/52	11.2	6.7	60	3.4			9.0	3.5	5.5	7.4	
			3/ 7/52	10.6	6.6	62	2.0			8.7	3.7	5.0	7.6	Course of ACTH i.m. 2/20 to 3/13/52. Total dose = 760 mg.
			3/17/52	10.4	6.6	63	3.4			8.8	3.8	5.0	7.7	
M. W.	A55193	Sickle cell anemia	1/24/52	16.5	10.6	64	6.0	5.9	98	11.7			7.5	
		Capillaria hepatica infestation of liver Vitamin D intoxication	2/ 7/52	14.6	10.5	72	4.8	5.5	115	12.0	3.0	9.0	7.6	

TABLE VII

Ultrafiltration of Serum from Patients with Hypoproteinemia

PATIENT	HISTORY NO.	DIAGNOSIS	DATE	SERUM Ca MG. %	FILTRATE Ca MG. %	% FILT. Ca	SERUM P MG. %	FILTRATE P MG. %	% FILT. P	SERUM TOTAL PROT. GM. %	SERUM ALBUMIN GM. %	SERUM GLOBULIN GM. %	SERUM NPN MG. %	FILTRATE PH	REMARKS
E. S.	473398	Nephrotic syndrome	4/17/52	7.6	6.3	83	6.6	7.1	108	3.5	1.0	2.5	48	7.3	
L. D.	532508	Disseminated lupus erythem.	9/27/50	7.2	6.0	83	4.9	5.4	110	4.4	1.4	3.0	60	7.4	
S. G.	590816	Idiopathic steatorrhea	12/ 1/51	8.5	6.5	76	3.5	3.8	109	4.8	3.3	1.5	33	7.4	
			12/20/51	9.4	6.7	71	4.8	5.0	104	5.9	3.8	2.1	31	7.7	After 19 days cortisone p.o. Total dose = 2 gm.
E. N.	610516	Nephrotic syndrome. Diabetes mellitus	7/ 3/52	8.8	6.6	75	6.3	6.8	108	4.8	2.2	2.6	80	7.4	
C. H.	547902	Arteriosclerotic nephritis	9/ 8/50	7.5	5.1	68	7.6	8.9	117	5.5	3.2	2.3	80	7.6	
C. C.	630487	Chronic glomerulonephritis with osteitis fibrosa cystica	2/ 3/53	10.2	7.4	73	9.5	10.3	108	5.9	3.9	2.0	154	7.3	Alkaline phosphatase = 12.5 B.U.*

* Bodansky units.

low percentage of ultrafilterable Ca in the presence of a reduced percentage of ultrafilterable P, both of which returned to the normal range with fall in the serum Ca and P, suggests the presence of a "colloidal calcium-phosphorus-protein complex" as mentioned before.

Group VII—Patients with Hypoproteinemia.

Sera from six patients with hypoproteinemia were ultrafiltered (Table VII). All except one of these patients had renal insufficiency of variable degree with nitrogen and phosphorus retention. Hypocalcemia was present in most instances. It is noteworthy that the percentage of ultrafilterable Ca was markedly elevated in the two patients (E. S. and L. D.) with extremely low concentration of serum albumin (less than 1.5 gm.%). The percentage of ultrafilterable Ca was 75% or greater in all four patients whose total serum protein

TABLE VIII

Ultrafiltration of Serum from Patients Treated with Cortisone or ACTH

PATIENT	HISTORY NO.	DIAGNOSIS	DATE	SERUM Ca MG. %	FILTRATE Ca MG. %	% FILT. Ca	SERUM P MG. %	FILTRATE P MG. %	% FILT. P	SERUM TOTAL PROT. GM. %	SERUM ALB. GM. %	SERUM GLOB. GM. %	SERUM NPN MG. %	FILTRATE PH	REMARKS
J. T.	554595	Osteoporosis (?atypical Cushing's syndrome)	10/25/50	10.5	7.0	67	3.8	4.0	105	5.9	4.4	1.5	22	7.6	
			11/ 2/50	10.4	7.4	71	3.9	4.2	108	6.1				7.5	
			11/ 9/50	10.4	7.1	68	3.6			5.9	4.4	1.5	24	7.6	Cortisone i.m., 11/2/50 to 11/16/50, 100 mg. q.d.
			11/16/50	9.0	6.4	71	3.5			6.0				7.6	
J. C.	524037	Sarcoidosis	10/19/50	13.1	9.3	71	3.6	4.1	114	7.1	4.2	2.9	52	7.6	
			10/31/50	13.8	10.2	74	4.6	5.3	115	7.6	4.4	3.2	64	7.3	
			11/ 7/50	13.4	9.3	69	4.5			7.4			67	7.5	Cortisone i.m., 10/31 to 11/13/50, 100 mg. q.d.
			11/13/50	10.6	7.8	74	4.5			7.3			64	7.5	
			5/17/51	11.5	8.0	70	4.3			8.4	5.2	3.2	60	7.6	
J. E.	494297	Sarcoidosis	1/18/51	16.0	11.1	69	3.9	4.2	108	7.8	3.9	3.9	50	7.6	
			1/31/51	10.6	7.6	72	3.5			6.5				7.5	ACTH i.m., 1/18 to 1/29/51. Total dose = 700 mg.
R. H.	610287	Sarcoidosis	1/ 2/53	12.2	8.4	69	3.6	4.1	114	7.8				7.5	
			1/ 4/53	13 6	8.8	64	3.8	4.3	113	8.2	5.1	3.1	64	7.5	
			1/14/53	11.5	7.6	66	3.6	4.1	114	7.8				7.6	After 5 days of cortisone p.o. Total dose = 900 mg.
C. H.	586874	Reticulum cell sarcoma with skeletal metastases	10/11/51	15.4	11.2	73	2.6	2.5	96	6.0	3.3	2.7	28	7.5	
			10/17/51	14.2	7.8	55	4.3	4.2	98	7.6			29	7.6	
			10/20/51	21.2	14.0	66	5.6	5.1	91	7.8			48	7.5	
			10/22/51	21.0	14.7	70	4.6	3.9	85	6.4	3.2	3.2	79	7.6	ACTH i.v. 10/21 to 11/4/51. Total dose = 444 mg.
			10/24/51	17.6	13.3	76	4.0	4.1	102	5.6				7.5	
			10/26/51	14.0	9.0	64	3.2	3.1	97	5.7			71	7.5	
			10/30/51	11.9	8.7	73	2.8	3.1	111	6.1			42	7.6	
			11/ 5/51	16.6	12.1	73	3.9	3.8	98	5.7				7.6	Patient died 11/6/51.

concentration was less than 5 gm.%. Cortisone therapy of patient S. G. produced a remission of her steatorrhea with rise in serum total protein and serum Ca concentrations. Coincidently with these changes the percentage of ultrafilterable Ca fell slightly. The percentage of ultrafilterable P was normal in all of these sera despite the hyperphosphatemia.

Group VIII—Patients Treated with Cortisone or ACTH.

Ultrafiltration data on the sera of five patients treated with cortisone or ACTH are presented in Table VIII. In addition five other patients similarly treated have already been presented in Tables V, VI and VII. It may be noted

in Table VIII that one of these patients suffered from idiopathic osteoporosis, three from sarcoidosis and one from reticulum cell sarcoma with widespread skeletal metastases. All except the first had hypercalcemia. All had either normal or slightly low serum protein concentrations. The percentage of ultrafilterable Ca in the sera of all except one (C. H.) was consistently within normal range, as was the percentage of ultrafilterable P, and did not change significantly with the therapy, although the total serum Ca fell in each case. Patient C. H. with reticulum cell sarcoma developed extreme hypercalcemia and mild azotemia while under observation, with symptoms resembling those seen in parathyroid intoxication, viz., drowsiness, vomiting and oliguria. The hypercalcemia was temporarily reduced by ACTH therapy with coincident symptomatic improvement. The serum showed wide variation in protein con-

TABLE IX

Ultrafiltration of Serum from Patients with Miscellaneous Diseases

PATIENT	HISTORY NO.	DIAGNOSIS	DATE	SERUM Ca MG. %	FILTRATE Ca MG. %	% FILT. Ca	SERUM P MG. %	FILTRATE P MG. %	% FILT. P	SERUM TOTAL PROT. GM. %	SERUM ALB. GM. %	SERUM GLOB. GM. %	FILTRATE PH	REMARKS
J. A.	500651	Hodgkin's disease	8/18/50	16.0	11.4	71	3.9			6.1	3.3	2.8	7.6	
R. F.	596164	Hyperventilation tetany	1/25/52	9.8	6.7	68	3.5	4.2	120	6.8	4.6	2.2	7.4	
			1/30/52	9.9	6.5	66	3.2	3.5	109				7.64*	Blood drawn with patient in tetany following 5 minutes of hyperventilation.
D. L.	122153	Chronic nephritis	5/14/52	7.3	5.2	71	13.3	14.8	111	7.1	3.7	3.4	7.6	N.P.N. = 184 mg.%

* Venous blood was drawn without stasis and centrifuged under oil for 10 minutes. The serum was then separated off and set up for ultrafiltration immediately without having been saturated with 5% CO_2 in 95% oxygen.

centration, probably related to fluctuation in the water balance. Considerable fluctuation in the percentage of ultrafilterable Ca of her serum was also noted, related in part to variation in protein concentration, but perhaps also due to the formation and later disappearance of a "colloidal calcium-phosphorus-protein complex." Evidence in favor of this is that the percentage of ultrafilterable P of the serum was consistently low except in the sample showing the least hypercalcemia.

Group IX—Patients with Miscellaneous Diseases.

In Table IX are collected the ultrafiltration data on the sera of three patients with diagnoses of Hodgkin's disease involving bone, tetany due to hyperventilation, and chronic nephritis with marked hyperphosphatemia, respectively. It may be seen that in all of these conditions the percentage of ultrafilterable Ca and P of the sera was within normal limits. There was no significant change in total or ultrafilterable Ca in the serum of patient R. F. when he developed tetany from hyperventilation.

DISCUSSION

In a previous paper (8) it was demonstrated that when calcium salts are added to pooled human serum, there results an increase in calcium proteinate as well as ultrafilterable calcium of such a degree that a relatively constant ratio between the two is maintained. In the cases of hypercalcemia here presented, in which serial samples of serum with various calcium concentrations were ultrafiltered, it appears that a ratio exists between the ultrafilterable calcium and total serum calcium which likewise is relatively constant. This can only occur if the amount of calcium proteinate per gram of protein in the serum increases with the rise in serum calcium concentration. Such a relationship between protein and calcium has been found to exist with a variety of proteins including fresh Red Cross normal serum albumin (human) (lot # 3043-R-1) where in a water solution containing 5.4 gm.% of albumin, 1.3 mg. of calcium are "bound" per gram of albumin when the total calcium concentration of the solution is 16.8 mg.%, whereas 8.1 mg. of Ca are "bound" per gram of albumin when the total Ca concentration in the system is 252 mg.% (pH 7.2–7.5) (12). Similar results were obtained with five Cohn fractions,[3] although corresponding fractions obtained at a later date differed from the original ones in their Ca binding capacity (12). Therefore, the observation of Martin and Perkins that more calcium is bound per gram of albumin-nitrogen in the serum of patients with hyperparathyroidism as compared with normals is not surprising unless the total calcium concentration in each system was made identical, and these concentrations were not stated in their communication (13). Thus ***it need not*** be postulated that when hypercalcemia exists, some abnormal protein or mucoprotein must be present in the serum to "bind" the extra calcium, since if this were present in significant quantity, the ratio between ultrafilterable Ca and serum Ca would be lower in hypercalcemia than in normocalcemia and such is not the case in the majority of patients we have studied. It is true that the relative quantity of calcium which will pass the ultrafilter may be reduced at very high concentrations of serum Ca, but in these cases there is also relative reduction of ultrafilterable P. Previous experiments indicated that in the presence of normal concentration of serum P, this latter phenomenon occurred in artificial hypercalcemia when serum Ca concentration reached or exceeded 19 mg.% (8). However, in the disease states presented here, it is found to occur occasionally at lower serum Ca concentrations (12.8 mg.%—G. S.—Table II) (14.0 mg.%—C. H.—Table VIII). At present we have no adequate explanation for this. It is of interest that in the group of patients in whom the ultrafilterable P was less than 100% (Table II, G. S.; Table IV, H. W., Table VI, M. W.; Table VIII, C. H.),

[3] Obtained through the courtesy of Edwin J. Cohn, Director, University Laboratory of Physical Chemistry, Harvard University.

vomiting and mild azotemia were prominent clinical features. In such samples of sera the percentage of ultrafilterable Ca may or may not be reduced. The explanation for this reduced ultrafilterability of P and usually Ca in the serum in these patients is not entirely clear, but it is thought that a non-ultrafilterable "colloidal calcium-phosphorus-protein complex" may be formed, as was postulated by Smith who did ultrafiltration studies on the sera of dogs made artificially hypercalcemic (14). It is suggested that this colloidal complex may be responsible for some of the clinical symptoms noted in patients with marked hypercalcemia, such as in cases of parathyroid poisoning (15) and very severe vitamin D intoxication (16).

It has been pointed out by some authors (17) that the ionized calcium in the serum is usually elevated in hyperparathyroidism and that this finding is of diagnostic significance in this disease. One author (18) has stated that the diffusible calcium may be elevated in this disease in the presence of normal total serum calcium, and that in an occasional patient this may be of even greater diagnostic importance. We believe that the above impressions arise from a misinterpretation of the data of McLean and Hastings (1). In their report, these authors stated that "an increase in the calcium-ion concentration of the plasma is presumptive evidence of hyperfunction of the parathyroid glands." They admitted, however, that they had not had the opportunity to study patients with hypercalcemia caused by diseases other than hyperparathyroidism. But these authors clearly pointed out that the calcium-ion concentration (which accounts for most of the diffusible fraction) of the plasma is the resultant of an equilibrium between total calcium and total protein present in the plasma with any change in calcium-ion concentration observed representing simply the quantitative changes predicted by the mass law equation. Therefore, from this, one could anticipate calcium-ion concentration to rise with elevations in total serum calcium concentration and conversely expect calcium-ion concentration to fall with lowering of the total serum calcium, provided protein concentration remained unchanged. This has been demonstrated by other investigators (7, 19).

An examination of the results in Table II readily reveals an elevated ultrafilterable calcium in hyperparathyroidism, but only when the *total* serum calcium concentration is concurrently elevated. In patient L. W., who had only very slight hypercalcemia (11.2 mg.%), the ultrafilterable calcium was 7.4 mg.%, a value which must be considered to be within the normal range when compared with the results in Table I. In examining the data of all other patients with hypercalcemia of nonparathyroid origin reported herein, it is obvious that the ultrafilterable calcium is elevated in every instance. This includes those cases of hypercalcemia with greatly increased protein concentrations in the serum. It should be mentioned that if the total serum protein concentration is below normal, there is a relative increase in ultrafilterable

calcium in the presence of normocalcemia, but this is directly dependent upon the *hypoproteinemia* and not due to the specific disease state *per se*. From these findings, it is believed that the presence of an elevated ultrafilterable calcium is of no value in establishing the diagnosis of hyperparathyroidism.

In the hypercalcemia due to vitamin D intoxication, the ratio of ultrafilterable to total Ca was found to be normal except in one serum sample of patient H. W. (Table IV) where, as mentioned above, there is a decreased percentage of ultrafilterable Ca and P possibly due to "calcium-phosphorus-protein complex" formation. In the hypocalcemia due to hypoparathyroidism, the percentage of ultrafilterable Ca was also found to be normal and to remain relatively constant for each patient after treatment with vitamin D had caused a rise in the total serum Ca. Thus, in our experience, vitamin D appears to exert no direct effect on the ultrafilterable Ca of the serum *per se* since changes in this moiety parallel changes in total serum Ca in degree such that a relatively constant ratio between the two is maintained. This experience is in disagreement with that of Anning, Dawson, Dolby and Ingram (20) who reported that there is a rise in diffusible Ca in vitamin D intoxication which is detectable even before the rise in total serum Ca concentration. It is recognized that these investigators used a different method of ultrafiltration and pH determinations were not recorded.

It may be pointed out here that in the group of hypoparathyroid patients (Table III), poor correlation of the degree of tetany with either the total serum Ca or the ultrafilterable Ca was found to exist. Patient A. C. evidenced severe carpopedal spasm when her serum Ca was 6.1 mg.% and her ultrafilterable Ca was 4.3 mg.%, whereas patients A. M., O. H. and M. B. manifested only latent tetany as demonstrated by positive Chvostek and Trousseau signs when their serum and ultrafilterable Ca concentrations were lower than this.

The hyperproteinemia frequently found in multiple myeloma does not appear to cause reduced ultrafilterability of the serum calcium, and indeed the serum of one patient of this group (S. C., Table V) showed increase in the ultrafilterable calcium. The reason for this may be that the increased protein concentration is due to an increase in the globulin fraction which usually has a lower calcium binding capacity than the albumin fraction. The factors reported by McLean and Hastings to transform *normal* serum albumin and globulin to base combining units are 0.273 and 0.189, respectively (21). This has been indirectly confirmed by Miller studying serum and transudates (22) and recently stressed by Gutman (6c). Similar results were obtained when sera which were hyperproteinemic due to other conditions were ultrafiltered, except for that of patient E. B. (#517118, Table VI). The serum of this patient who suffered from chronic lymphoid leukemia was found to have a somewhat low percentage of ultrafilterable Ca. The serum was extremely viscid and could be recognized in the gross as being highly abnormal. Very likely and

as yet unidentified protein constituent of her serum was responsible for the increased viscosity as well as the increased amount of "bound" calcium. Unfortunately, no electrophoretic pattern of this serum was obtained.

In the past it has often been suggested that when hyperproteinemia is present with hypercalcemia the increased protein concentration accounted for the hypercalcemia. However, this has not been substantiated by some investigators (6c, 23). It should be noted that in our experience also (Tables V and VI), there is no correlation between the presence of hypercalcemia and hyperproteinemia, since each may exist independently of the other. Therefore, for purposes of clinical evaluation, hypercalcemia should be considered to reflect an abnormality of calcium metabolism even in the presence of marked hyperproteinemia. In only one instance in this series (E. B. #517118) (Table VI),

TABLE X

Ultrafiltration Data on Myeloma Serum with Added Calcium

T.P. = 9.6 gm.% Albumin = 2.4 gm.% Globulin = 7.2 gm.%*

SAMPLE	TOTAL Ca MG. %	FILT. Ca MG. %	% FILT. Ca	TOTAL P MG. %	FILT. P MG. %	% FILT. P	FILTRATE PH
1	13.4	10.8	81	3.9	4.1	105	7.6
2	16.3	12.7	78	3.9	4.0	102	7.5
3	18.9	14.8	78	4.1	3.8	93	7.6
4	21.0	16.9	80	3.9	3.5	90	7.5
5	25.1	17.4	69	3.8	2.7	71	7.6

To 10 cc. aliquots of serum from patient S. C. (Table V) were added 0, 0.2, 0.4, 0.6 and 0.8 cc. of a 0.4% $CaCl_2$ solution. Sufficient normal saline was added to make the final volume of each sample 11 cc. A portion of each sample was analyzed for Ca and P. The remainder of each sample was saturated with 5% CO_2 in 95% O_2 and subjected to immediate ultrafiltration.

* Calculated values, based on 10/11 of the undiluted serum concentrations.

where hypercalcemia and hyperproteinemia coexisted, was the ultrafilterable calcium normal (as expressed in mg.%). In this patient's serum, there was present a highly unusual protein which apparently "bound" an abnormal amount of calcium, as noted above.

When hypocalcemia is met, however, the serum protein should always be determined before evaluating the significance of the hypocalcemia; for in all the patients with severe hypoproteinemia (serum total protein less than 5.0 gm.%) herein reported, the ultrafilterable calcium of the serum (in mg. %) was either normal or very close thereto.

We believe it is of interest at this point to mention some studies performed on patient S. C., who had multiple myeloma and hypercalcemia (Table V). The hypercalcemia was further increased *in vitro* and ultrafiltration studies carried out (Table X). Serum P and protein concentrations were kept constant. It can be seen that the percentage of ultrafilterable Ca, which was ini-

tially quite high, remained essentially unchanged until a serum Ca level between 21 and 25 mg.% was reached, at which point there was a sharp reduction in ultrafilterable Ca. Ultrafilterable P, on the other hand, was reduced at a serum Ca level of 18.9 mg.% and continued to decrease as serum Ca was increased, again probably due to the formation of a "colloidal calcium-phosphorus-protein complex" as discussed above.

In patients with hypoproteinemia, the percentage of ultrafilterable Ca is somewhat higher than in the group with normal concentrations of serum proteins at similar levels of total serum calcium. This finding may help to explain why tetany is seldom seen in patients with hypocalcemia due to hypoproteinemia, since the ultrafilterable Ca is actually within or near the normal range. In this regard, we realize that in the hypocalcemia of chronic renal disease, the frequently accompanying acidosis is also an important factor in preventing tetanic symptoms.

The patients with uremia presented herein, who demonstrated hyperphosphatemia in the absence of hypercalcemia, have normal percentages of ultrafilterable P. This is in agreement with the results of previous ultrafiltration studies on pooled human serum made artificially hyperphosphatemic *in vitro* (8).

The effect of ACTH or cortisone therapy in several disease states was of great interest. Three patients with hypercalcemia due to sarcoidosis (Table VIII) were treated in this manner and return to normocalcemia occurred within two weeks in all instances. (Serum from patient R. H. was not obtained for ultrafiltration after normocalcemia was established). Three patients with hypercalcemia due to multiple myeloma were treated with ACTH (Table V) and normocalcemia became apparent within two weeks in two of the patients. The third (J. W.) was not studied sufficiently to determine whether this occurred. A patient with severe hypercalcemia due to reticulum cell sarcoma with skeletal metastases (Table VIII, C. H.) was treated with ACTH and a prompt fall in serum Ca to nearly normal concentration with improvement in clinical symptoms was noted. However, as the ACTH dosage was tapered off and then stopped, hypercalcemia promptly recurred. Return to normal of the mildly elevated serum Ca occurred in patient E. B. (#517-118—Table VI) who was receiving ACTH therapy for chronic lymphoid leukemia. The mechanism of reduction of hypercalcemia by means of ACTH or cortisone therapy is unknown, and no hypothesis is offered at present since metabolic balance studies were not carried out on these patients. A patient with osteoporosis (probably atypical Cushing's syndrome) (J. T., Table VIII) was treated with cortisone and a slight fall was observed in his serum Ca, not attributable to hemodilution since serum protein concentration remained essentially unchanged. Urine calcium excretion also was reduced slightly

during this period of cortisone therapy. Patient S. G. (Table VII) with hypocalcemia and hypoproteinemia due to idiopathic steatorrhea manifested a *rise* both in serum Ca and serum protein concentrations after 10 days of cortisone therapy, during which time a remission in her disease occurred. Similar responses have been noted by others in this disease (24).

From the ultrafiltration studies presented here on the serum of patients treated with ACTH or cortisone, it appears that these drugs exert no direct effect on the ultrafilterable Ca and that any changes occurring in this fraction of the serum Ca are related to the changes in total serum Ca or serum protein (or some fraction of the latter). When no change takes place in protein concentration, the ratio between ultrafilterable Ca and total serum Ca remains relatively constant, despite reduction in total serum Ca.

In the patient with tetany due to hyperventilation (25), it is not surprising that there is no difference in the percentage of ultrafilterable Ca of his serum while in tetany as compared with a control value, since the alkalosis induced by brief hyperventilation is of such slight degree (arterial blood pH change of 7.39 to 7.55 in the case of R. F., Table IX) as to cause a change in percentage of ultrafilterable Ca less than the error of the method (8). McLean and Hastings also stated that "the tetany of alkalosis does not appear to be associated with diminished concentrations of Ca^{++} in the plasma" (1).

Thus it seems apparent that the relationships between calcium, phosphorus and protein in pathological sera are similar to those found in pooled human serum made abnormal artificially *in vitro* (8). When the pH during ultrafiltration is kept within reasonably physiological limits, any significant change in the percentage of ultrafilterable Ca or P appears to be purely secondary to a change in total serum Ca concentration (or to a lesser extent to serum P concentration), or to a change in the concentration or types of serum proteins. This permits one to predict with reasonable accuracy the percentages of ultrafilterable Ca and P in serum (provided normal concentration of protein constituents is present) within a range of serum Ca concentrations of 4 to 13 mg.%. Above a serum Ca concentration of 19 mg.%, if the serum P is normal, or between a serum Ca of 13 and 19 mg.% if the serum P is elevated, one can predict decreased ultrafilterability of serum P and perhaps also of serum Ca with the associated clinical symptoms of vomiting, lethargy and oliguria. With exceedingly rare exceptions (see leukemia case, Table VI), serum with elevated globulin concentration will in general have a normal or even slightly elevated percentage of ultrafilterable Ca, and serum with a low protein concentration will have an elevated percentage of ultrafilterable Ca which will be roughly in inverse proportion to the protein concentration. This predictability negates the usefulness of ultrafiltration as a diagnostic procedure and renders it helpful only as an experimental tool in determining the relationships of the various serum components.

SUMMARY AND CONCLUSIONS

1. Ultrafiltration data are presented using Lavietes' technique allowing rigid pH control on sera from patients with hyperparathyroidism before and after removal of parathyroid adenomas, hypoparathyroidism before and after vitamin D therapy, vitamin D intoxication, multiple myeloma and other diseases associated with hyperproteinemia, sarcoidosis, idiopathic osteoporosis, sprue, malignancy, renal disease and tetany due to hyperventilation.

2. No correlation was found to exist between hypercalcemia and hyperproteinemia in the disease states studied. Hypoproteinemia was in general associated with hypocalcemia.

3. An elevated ultrafilterable calcium is of no differential diagnostic value in hyperparathyroidism.

4. The inorganic phosphorus of serum was for all practical purposes wholly ultrafilterable except in cases of marked hypercalcemia (or moderate hypercalcemia with concomitant hyperphosphatemia) wherein it was reduced and associated with mild azotemia, vomiting, drowsiness and oliguria in these patients.

5. The ratio of ultrafilterable Ca to total serum Ca was in general found to be within the range of the normal subjects except under these circumstances: (a) Some instances of hyperglobulinemia when the ratios tended to be elevated. (b) Hypoproteinemia of less than 5 gm.% where the ratios were also elevated. (c) Marked hypercalcemia (*vide supra*) where the ratios were sometimes low associated with decreased ultrafilterability of P possibly due to formation of "a non-ultrafilterable Ca-P-protein complex."

6. The ratio between ultrafilterable Ca and total serum Ca remained relatively constant despite marked changes in total serum Ca concentration induced by ACTH, cortisone and vitamin D therapy and surgical correction of hyperparathyroidism.

7. Cortisone and ACTH therapy caused reduction of hypercalcemia without change in the ratio between ultrafilterable calcium and total serum calcium in three cases of sarcoidosis, two of multiple myeloma and one of reticulum cell sarcoma.

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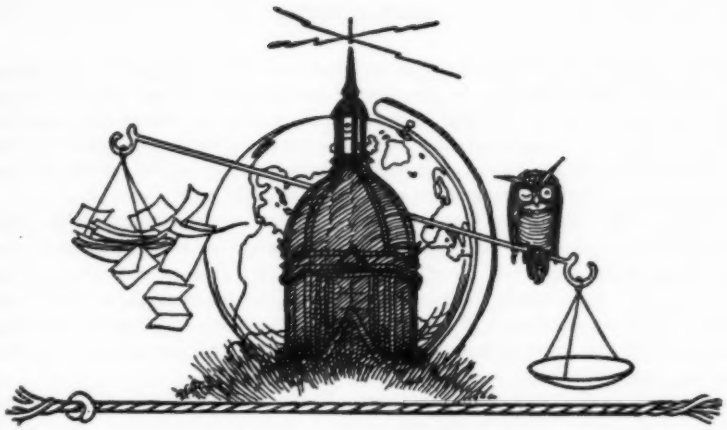

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Synopsis of Pathology, 3rd edition. By W. A. D. ANDERSON. 788 pp., $8.00. The C. V. Mosby Co., St. Louis, Missouri.

For many years Dr. W. A. D. Anderson's pathology text, *Synopsis of Pathology*, has enjoyed a well-earned popularity among second year medical students. This book now appears in its third edition. The author has dealt admirably with the subject matter for the most part and its concise presentation of the cardinal features of disease entities would seemingly give the student a frame-work on which to build his understanding of pathology. The photographs illustrating gross and microscopic characteristics of diseased tissue are excellent.

In a small text-book that deals with a broad subject such as pathology there always arises the problem of how much space should be given to various disease entities. Dr. Anderson includes in this text rather comprehensive discussions of tumors. From my own viewpoint I feel that the limited space could have been put to better use in more comprehensive description of such diseases as nephritis, and heart disease, with perhaps a second volume dealing with tumor pathology. In teaching second year medical students, in general, we cannot devote very much time to the specialized field of tumor pathology.

STANTON L. EVERSOLE, JR.

Elementary Medical Statistics. The Principles of Quantitative Medicine. By DONALD MAINLAND. 327 pp., $5.00. W. B. Saunders Co., Pa.

The author is Professor of Medical Statistics at New York University College of Medicine and was formerly Professor of Anatomy at Dalhousie University. The book is based on lectures given at Dalhousie to first-year medical students, with some supplementary material, but is intended also for medical investigators.

The book opens with a reference to the comparative neglect of sound statistical methods in medical research; a neglect which the author imputes in part to the nature of medical education, with its emphasis on memorization and techniques. Chapter II, "On looking at

evidence," outlines the principal types of variation and error to which data may be subject and notes the implications for the planning of investigations. Count data and ratios derived from them (Chapter III) lead to the comparison of ratios and to some useful advice about the planning of experiments designed to compare two methods of treatment (Chapter IV). Then follows the analysis of continuous measurements and of the relationship beween measurements, as studied by regression and correlation (Chapters V and VI).

In Chapter VII, "Statistical ideas in clinical practice," the author applies the principles and techniques developed in previous chapters to an appraisal of three topics with which most clinicians are concerned, directly or indirectly: the concept of a "normal" and of "normal limits": the errors in routine laboratory analyses (chemical, blood counts, glucose in urine, X-ray measurements of the heart); and the routine statistics of morbidity and mortality. Notes on more advanced techniques, including an introduction to the analysis of variance, are given in the final chapter.

Problems in medical research and numerous illustrations from medical data are used to introduce and lighten the technical exposition. A series of questions at the end of each chapter should stimulate the thinking of the reader. The writing is, in general, clear and easy to follow. On one point the author seems confusing. On the analysis of paired data, when the pairing does not appear to have been successful, he writes (p. 158) "Therefore, if the mean of a series of differences is not significant, we may test the difference between the means (but see p. 113)". What particular passage on p. 113 is implied is not stated, but this page does contain the contradictory statement "a second test of the same hypothesis with the same data is not permissible."

The book covers roughly the same ground as Bradford Hill's deservedly popular *Principles of Medical Statistics*. Since students of statistical methods show marked individual preferences for books, despite learned advice from their professors, a comparison of the relative merits of the two works seems scarcely profitable. Both books are good.

W. G. COCHRAN

Encyclopedia of Aberrations. Edited by EDWARD PODOLSKY. With a Foreword by Alexandra Adler. 550 pp., $10.00. Philosophical Library, New York.

This is an alphabetically arranged miscellany containing reprinted journal articles, short statements and brief, dictionary-like definitions of a heterogeneous variety of psychopathic and sociopathic phenomena. There is no detectable plan of selection unless it be to emphasize the unusual and sensational at the expense of the important. There are long case studies of trivial or rare conditions such as the "syndrome of Capgras", of which 7 cases have been reported, "chloral delirium" and "folie a deux". "Post-orgastic emptiness, fear of" receives 9 pages. Yet such topics as barbiturate intoxication and neurosis, are not even listed, and "obsessive-compulsive behavior" and "hysteria" get less than a page each. The level of the articles varies widely and some are excellent. However, the selection is too capricious and the quality of the contributions too uneven to make this a useful reference work, though it is satisfactory for browsing purposes.

JEROME D. FRANK

A Translation of Galen's Hygiene (De Sanitate Tuenda). By ROBERT MONTRAVILLE GREEN. 277 pp., $5.75. Charles C Thomas, Springfield, Illinois.

This successful labor of love by Dr. Green is introduced by a felicitous appreciation of Galen by Dr. Henry Sigerist. In it he distills the conflicting reactions evoked in one who reads this treatise for the first time. From the complacency of this present time one looks back across almost 22 centuries and is moved alternately by feelings of superiority and of

admiration. Even though one may wonder in fairness whether the connotation of some words has so changed as to make impossible a clear understanding of what Galen meant, nevertheless, some of his ideas appear so ridiculous that today no more than several million faddists believe them. And in the next moment, with the same reservations about meaning, one is convinced that Galen has expressed an immutable truth. Thus it is an entertaining work, even for those of us whose familiarity with the times in which he wrote is meager to say the least. Whether Galen discusses exercise and massage, forms and treatment of fatigue, emaciation and obesity, or the theory of therapeutics there is amusement and not a little wonder here for anyone who reads it. In some ways it suggests a fantastic joint effort by the editors of *Physical Culture* and the *American Journal of Hygiene*. Dr. Green has provided an extraordinarily graceful translation for what is essentially a ponderous Galenical style. It has become the mode to complete a review with some statement describing whose library shelves should contain the book in question. Galen's *Hygiene* sits well on any shelf, and you can take your time about reading it: it has been waiting for you for over 2100 years.

JOSEPH L. LILIENTHAL, JR

The Photography of Patients. (American Lecture Series). By H. LOU GIBSON. 118 pp., $5.50. Charles C Thomas, Springfield, Illinois.

This volume of the American Lectures in Medical Photography is edited by Ralph P. Creer, F.B.P.A. Containing considerable practical information for the beginner and the physician who wishes to make photographic records of patients in his office, this book is also an excellent review for the experienced institutional photographer.

A chapter on Equipment and Space offers sound advice on selection of equipment for and establishment of a medical photographic department. Following this is a chapter devoted to the use of roll film cameras with supplementary lenses and focal frames. This chapter is intended for the amateur or physician wishing to utilize his personal picture taking equipment. There are numerous diagrams and tables of technical data indicating proper exposure with given working distances and lens apertures. This information should prove invaluable to the amateur wishing to avoid the more laborious trial and error methods.

Professional and amateur photographers alike may profit from the advice given in the chapter on Lens Properties. The aspect of perspective as related to focal length of lenses should receive serious consideration, especially by those making plastic surgery progress records. Uniformity of procedure in posing of patients is recommended and several standardized lighting plans shown in diagrams are covered in the chapter on Positioning and Lighting. Comparison photographs demonstrating correct and incorrect approach to the lighting of clinical subjects are included.

Chapter VI discusses the physical characteristics of photographic films and their use with filters. Films made by several manufacturers are listed with their specific application. Included are three exposure guide tables for use with floodlamps, flashlamps and close-up flashlamp photography. The two chapters entitled Printing Methods and Paper Selection, Quality Control and Print Quality contain advice for those wishing to produce their own prints of high quality by contact or enlargement. This is an important step in the production of good photographic illustrations. The inherent quality of many excellent negatives is lost through inadequate print control.

This book is well illustrated with many photographs and diagrams adapted from articles published in *Medical Radiography and Photography*. The quality of reproduction is extremely good. This book is an excellent guide for the beginner and those teaching medical photography.

CHESTER F. REATHER

Group Psychotherapy. Studies in Methodology of Research and Therapy. Report of a Group Psychotherapy Research Project of the U. S. Veterans Administration. By FLORENCE B. POWDERMAKER AND JEROME D. FRANK. 615 pp., $6.50. Harvard University Press, Cambridge, Mass.

In the specialty of psychiatry at the present time numerous schools of psychiatric theory and practice have arisen but the actual body of established factual knowledge is still small. Any attempt at systematic, objective study of psychotherapy is therefore noteworthy and laudable. The present volume represents an important contribution in this direction. It is published as a progress report of a study in psychotherapy financed by the U. S. Veterans Administration and carried out under the auspices of the Washington School of Psychiatry during the years 1947–1949. The primary focus of this study was on interpersonal events occurring in the course of active therapy, in a group setting, with neurotic and schizophrenic patients. The primary aims of the study were to obtain data as objectively valid as possible and to formulate these data along qualitative lines. Twenty-four psychiatrists, five clinical psychologists, and two psychiatric social workers collaborated in this project. It was found that data obtained by specially trained observers from this professional group, working in close collaboration with the psychiatrists in charge of the group meetings with patients constituted the most reliable objective findings. The important role of the observer as the instrument for recording objectively valid data is considered in some detail. The accumulation of such observations led to a method of formulating the clinical data called situation analysis. A situation was found to consist of a pattern of events, with some action of one individual producing an effect on another or others, and subsequent events representing a direct consequence. When many such situations were so analyzed, patterns of apparent similarity in form were found.

This research project was divided into two sections and each is reported separately in the book. Part I, Group Therapy with Clinic Patients, presents observations on neurotic patients treated in group therapy as out-patients at the Veterans Administration Mental Hygiene Clinic in Washington, D. C. Part II, Group Therapy with Hospitalized Schizophrenic Patients presents studies of chronic schizophrenic patients who had not responded to routine hospital care or shock treatment at the Veterans Administration Hospital at Perry Point. Situation analyses make up the core of the chapters in which therapeutic problems and clinical events are discussed, and 63 in all are presented. Various methods for evaluating patients are considered in detail in six appendixes. There is a list of references and an index.

BARBARA J. BETZ

Functional and Surgical Anatomy of the Hand. By EMANUEL B. KAPLAN. 288 pp., $10.00. J. B. Lippincott Co., Philadelphia, Pa.

This book is unique in its field. It is divided into three portions. Part I is devoted to The Hand as an Organ in which the positions and fundamental functions of the hand are described, illustrated and discussed in detail. There are valuable tables on the functional length of the different tendons and a good discussion of the mechanism of coordinated action. There is also a table of the time of appearance of the different ossification centers.

Part II is concerned with the Structure and Function of the individual parts of the hand. These are described and illustrated in great detail and present a clearer picture of the precise anatomy and mechanics of function of these parts than the present reviewer has previously encountered.

Part III is devoted to the Surgical Anatomy which includes the surface anatomy of the hand and wrist, the surgical approaches for operations on each individual portion of the

hand and wrist, and finally, sections on amputations and reconstruction operations, all of which are well illustrated.

The book is primarily, as its title implies, one in which the detailed anatomy of the hand and wrist is presented and correlated with its function. The author does not attempt to present surgical technics nor compare clinical results of various operative procedures. It is not an "Operative Surgery", but is an excellent supplement to one.

The text is well written and the descriptions are clearly and concisely presented. The illustrations are nearly all original and demonstrate the anatomy and function of the hand and wrist in an often new and instructive way. Much fundamental investigative work is included.

This reviewer recommends the book for those surgeons who regularly or occasionally are called upon to treat injuries or disabilities of the hand. Its value is as a reference for the anatomy and normal and abnormal function of that structure. The information obtained from it will aid greatly in making an accurate diagnosis of the disability present and in selecting the proper operative procedure for treatment.

For a surgeon undertaking an unfamiliar operation on this area it will need to be supplemented by reference to an "Operative Surgery" for a detailed description of the technic. The book should also be valuable to the teacher of anatomy.

RICHARD T. SHACKELFORD

Treatment of Mental Disorder. By LEO ALEXANDER. 507 pp., $10.00. W. B. Saunders Co., Philadelphia, Pa.

This is an extremely useful compendium of the varieties of treatment employed in psychiatric illness. It is primarily written for those about to begin their psychiatric hospital training, general practitioners and students. For them, it presents an intensive, well-documented coverage of the physical and psychotherapeutic approaches that make up the psychiatrist's armamentarium. In this respect particularly it will prove to be a very valuable reference source. It also makes available reports of more than 38,000 cases of mental disorders culled from the literature. These have been organized in terms of the effectiveness of therapeutic techniques, in itself a commendable accomplishment, and serve as a frame of reference from which to view therapy in general. Though one may take issue with the lack of rigorous standards in the multiple reports referred to, the author is aware of this and presents the data as the best available at this time. However he is inclined to use them as a more infallible basis for the determination of which treatments should be employed in certain types of illnesses than is warranted.

In terms of emphasis, most of it is placed upon the description and utilization of physical treatments. This is unfortunately necessary, since psychotherapy *per se* is a subject that does not lend itself easily to relatively brief survey, nor is it something that can be prescribed or manipulated in the manner sometimes implied in the text. The description of psychotherapy and its varieties of kinds is short, dogmatic and useful primarily as a didactic introduction. However, his discussion of the physical treatments is extremely well done and accurate. It enables the inexperienced not only to learn what these are about, but almost in the nature of a handbook, permits him to follow stepwise the techniques in the administration of these. The chapters devoted to this topic are replete with valuable information including such things as necessary equipment, patient preparation, complications, the prevention of emergencies and their management should they occur. The special usefulness of each of the treatments and their indications are well covered. The field of electrical treatment is elaborated much too extensively with the result that one is left with overly specific ideas as to efficacy and a feeling that the ultimate in therapy has been reached here. How-

ever, at best these, like the rest of physical therapies, are empirical and occasionally are effective where one would not expect this and vice versa. Speculations are offered as to the *modus operandi* of the physical therapies as well as to the possibility and nature of damage to the central nervous system incident to their employment. Quite apart from this, the wide range of physical treatments, some usually only known about generally and practiced relatively infrequently, are covered very adequately. The author presents some interesting ideas on the importance of combined psychotherapy and physical therapy. However in his discussion of this he indirectly and unjustifiably limits the application of the former from which so many rich contributions to the understanding and treatment of mental illness have and will continue to come. There also is a sensing here as well of an unduly specific manipulation and combination of treatments which does not seem warranted.

Other very useful portions of this volume are the chapters given over to the treatment of alcoholism, organic cerebrospinal disease and states of intoxication. The last mentioned is particularly well executed.

A separate chapter is devoted to the nurse in the management of psychiatric illness. This is well done especially in relation to physical treatments but could have been expanded to include more about their importance in psychotherapy.

Included as well is a remarkably exhaustive bibliography which will also prove to be very valuable.

There is no doubt that this book will be widely read and quite eminently fulfils the purpose for which it was written. It seems likely to have an audience much larger than that for which the author modestly intended it.

LESTER H. GLIEDMAN

BOOKS RECEIVED FOR REVIEW

Aviation Toxicology. An Introduction to the Subject and a Handbook of Data. Prepared under the direction of the Committee on Aviation Toxicology, Aero Medical Association. Edited by C. R. SPEALMAN. 120 pp., $4.00. The Blakiston Co., New York.

Conferences on Drug Addiction Among Adolescents. Sponsored by the Committee on Public Health Relations of the New York Academy of Medicine with the assistance of The Josiah Macy, Jr. Foundation. Conferences held at the New York Academy of Medicine, November 30, 1951 and March 13 and 14, 1952. 320 pp., $4.00. The Blakiston Co., New York.

Cybernetics. Transactions of the Ninth Conference, March 20 and 21, 1952. Edited by HEINZ VON FOERSTER. 184 pp., $4.00. The Josiah Macy, Jr. Foundation, New York.

Experimental Studies in Psychiatric Art. By E. CUNNINGHAM DAX, with a foreword by Sir Steuart Wilson. 100 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

Mechanisms of Urologic Disease. By DAVID M. DAVIS. 156 pp., $4.50. W. B. Saunders Co., Philadelphia, Pa.

The Pharm-Assist Manual (Formerly Gray's Pharmaceutical Quiz Compend). Completely Rewritten by A. E. SLESSER. With a Foreword by Earl P. Slone. 167 pp., $3.50. The C. V. Mosby Co., St. Louis, Missouri.

Rehabilitation of the Physically Handicapped. Revised edition. By HENRY H. KESSLER. 275 pp., $4.00. Columbia University Press, New York.

The Roots of Psychotherapy. By CARL A. WHITAKER AND THOMAS P. MALONE. 236 pp., $4.50. The Blakiston Co., New York.

The Sulphonomides and Antibiotics in Man and Animals. By J. STEWART LAWRENCE AND JOHN FRANCIS. 482 pp., 42s. net. H. K. Lewis & Co., London, England.